PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 4705-PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/07564	International filing date *(day/month/year)* 22 MARCH 2000	Priority date *(day/month/year)* 22 MARCH 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant The BOARD OF GOVERNORS FOR HIGHER EDUCATION, STATE OF RHODE ISLAND AND PROVIDENCE PLANTATIONS		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of report with regard to novelty, inventive step or industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand 18 OCTOBER 2000	Date of completion of this report 16 MAY 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BENNETT CELSA Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998) ★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/07564

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-52, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of ______

[X] the claims:
pages 53-63, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of ______

[X] the drawings:
pages 1-6, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of ______

[X] the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of ______

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language ______ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

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III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 6 AND 8

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _ are so unclear that no meaningful opinion could be formed *(specify)*.

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 6 AND 8.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
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V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-4 AND 7	YES
	Claims	5	NO
Inventive Step (IS)	Claims	3-4	YES
	Claims	1-2, 5 AND 7	NO
Industrial Applicability (IA)	Claims	1-5 AND 7	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claim 5 lacks novelty under PCT Article 33(2) as being anticipated by WO 95/04277. This document discloses (e.g. on page 6, Formula 1) compounds which encompass the presently claimed formula (12) compounds when in the reference formula I: M1 is a bond; X1, Y1, Z1 are N and 2 methylenes; and A4 and R4 are substituted acyl compounds e.g. A4 is CoC(O)NR21 or when absent R4 is similar to A4 substituent. In this regard, this claim is a product-by-process claims which is being treated as a product claim. Accordingly, this reference discloses formula (12) libraries within the scope of the presently claimed invention, regardless of method of making.

Claims 1, 2 and 7 lack an inventive step under PCT Article 33(3) as being obvious over Tam ('356), Ogino et al. ('387), Flynn ('502), Turchi et al., Edwards et al ('371), and WO 95/04277.

Present claims (e.g. 1 and 2) address methods of making N-protected thiazole/oxazole amino acids by condensing an alpha thiol or alpha hydroxy amino acid (e.g. cys or ser), respectively with a glycaldehye or glycimine intermediate following by the oxidiation of the resulting thiazolines or oxazolidines to form protected/unprotected oxazoles/thiazoles

The condensation of aldehydes (E.g. amino acid aldehydes) with alpha amino acid thiols or alcohols to form thizaolidines is known in the art. See e.g. Tam at Fig. 1; Flynn at col. 7, Scheme B; Ogino et al. Similarly, oxazolidines can be made by condensation via an iminoether intermediate. See e.g. Turchi section 1.210.

Means to oxidize thiazolines to thiazoles and oxazolidines to oxazoles is well known in the art. E.g. See Edwards et al.(371) and Turchi et al.

The above references differ from the presently claimed invention by failing to disclose the use of the resulting oxazoles/thiazoles as intermediates (e.g. scaffolds) for making libraries

(Continued on Supplemental Sheet.)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): C07D 277/04, 277/08, 277/20, 277/22, 277/28, 277/30, 275/02, 263/30, 263/34; C12Q 1/00 and US Cl.: 548/146, 200, 201,202, 203, 204, 205, 214, 235, 236; 435/4, digest 34,

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

However, the WO 95/04277 reference teaches the beneficial use of oxazoles/thiazoles as scaffolds in libraries for screening pharmaceutically active compounds. Eg. see abstract, claims and formula I compounds on page 6.

Accordingly, the formation and use of thiazoles or oxazoles made by use of conventional reaction schemes as described above would have been obvious to one of ordinary skill in the art since the WO 95/04277 provides motivation to make screening libraries comprising thiazole/oxazoles as scaffolds.

Claims 3 and 4 meet the criteria set out in PCT Article 33(2)-(4), because the prior art of record does not teach or fairly suggest the making of fused oxazoles/thiazoles as described in these claims.

------------------- NEW CITATIONS -------------------
NONE

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
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ML	Mali
MN	Mongolia
MR	Mauritania
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VN	Viet Nam
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ZW	Zimbabwe

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 September 2000 (28.09.2000)

PCT

(10) International Publication Number
WO 00/56724 A1

(51) International Patent Classification[7]: C07D 277/04, 277/08, 277/20, 277/22, 277/28, 277/30, 275/02, 263/30, 263/34, C12Q 1/00

(21) International Application Number: PCT/US00/07564

(22) International Filing Date: 22 March 2000 (22.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/125,501 22 March 1999 (22.03.1999) US

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(81) Designated States (*national*): CA, JP, US.

(84) Designated States (*regional*): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *with international search report*

(48) Date of publication of this corrected version:
25 October 2001

(15) Information about Correction:
see PCT Gazette No. 43/2001 of 25 October 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/56724 A1

(54) Title: OXAZOLE AND THIAZOLE COMBINATORIAL LIBRARIES

(57) Abstract: This invention provides a novel method for synthesizing an ensemble of peptides that allows for the generation of an unlimited number of antibiotic compounds. More specifically, the method comprises utilizes synthetic heterocyclic amino acides containing thaizole and/or oxazole as building blocks in a solid phase combinatorial synthesis to yield natural and unnatural antibiotic compounds.

Title of the Invention

OXAZOLE AND THIAZOLE COMBINATORIAL LIBRARIES

Background of the Invention

1. Field of the Invention

This invention relates to the syntheses of thiazole and/ or oxazole-containing amino acids and more specifically to the use of those compounds in a combinatorial synthesis to generate antibiotic compounds.

2. Description of the Related Art

More and more thiazole and/or oxazole-containing peptides with important biological activities such as antitumor, antifungal, antibiotic, and antiviral activities have been found from microbial and marine origins. It seems that the thiazole and oxazole ring systems might be important pharmacophores in those biologically active compounds.

Bleomycin A_2 is a clinically used antitumor drug. Antibiotic GE 2270A is a novel inhibitor of bacterial protein synthesis. Antibiotic A 10255 factor B is a bacteriocide. Trioxazole-containing macrolides ulapualides, kabiramides, halichondramides, myalolides and jaspisamides show antifungal activity. Moreover, ulapualides inhibit L1020 leukemia cell proliferation and halichondramides inhibit cell division. Tantazole A is a member of a unique family of mirabazoles and tantazoles which show selective toxicity against solid tumors, and thiangazole is a novel inhibitor of HIV-1. BE 10988, a potent inhibitor of topoisomerase II, inhibited the relaxation of pBR322 plasmid DNA by topoisomerase II and significantly inhibited the growth of adriamycin and vincristine resistant P-388 murine leukemia as well as sensitive P-388 cell line.

Microcin B17, a peptide antibiotic with four thiazole and four oxazole rings, induces double-strand cleavage of DNA in a DNA gyrase-dependent reaction.

More interestingly, Escherichia coli *sbm*A mutants, which lack the inner membrane protein (SbmA) involved in microcin B17 uptake, were found to be resistant to bleomycin.

The traditional synthesis of biologically active compounds, such as compounds comprised of thiazole and/or oxazole compounds, has involved the optimization of a lead compound, usually derived from biological sources. The optimization process through traditional synthesis, purification, characterization and screening is lengthy, painstaking

and expensive. With the need to find more efficient methods of drug discovery and the advances in molecular biology and gene technology resulting in "high-throughtput screening", combinatorial synthesis represents a new method to simultaneously generate many different compounds with defined structures to accelerate the search for new lead compounds and their optimization (including their structure-activity relation).

Combinatorial synthesis can be performed either in solution or on solid phase. Solid phase synthesis was introduced by R. B. Merrifield in an effort to overcome many problems of peptide synthesis in solution. In 1963, Merrifield published the first solid phase synthesis of a tetrapeptide in Merrifield, *Solid Phase Synthesis Peptide Synthesis: The Synthesis of a Tetrapeptide*, Journal of the American Chemical Society **85**, 2149-2154 (1963). Today, the development of solid phase synthesis has extended to the syntheses of other biopolymers such as polynucleotides and polysaccharides, recently to the synthesis of small organic compounds and combinatorial synthesis.

Solid phase peptide synthesis is based on the attachment of α-amino and side-chain protected amino acid residues to an insoluble polymeric support (usually resin in peptide synthesis), followed by stepwise addition of protected amino acids to assemble the peptide chain on the solid support. After the attachment of the first α-amino and side-chain protected amino acid residue to the resin and the removal of the α-amino protecting group, a second α-amino and side-chain protected amino acid residue is attached to the free amino group of the resin-bound amino acid through the formation of an amide bond under the activation of a coupling reagent. Through this cycle, a planned peptide sequence can be assembled on the resin. Finally, the synthesized peptide chain can be cleaved from the resin and the side-chain protecting groups on the amino acid residues were removed simultaneously to obtain the expected peptide.

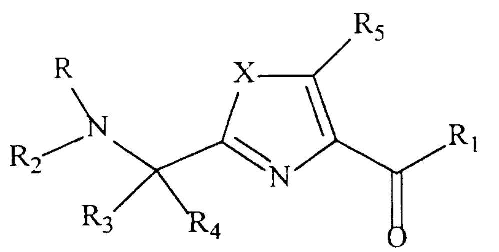
The present invention provides a novel method for the production of biologically active compounds comprised of thiazole and/or oxazole ring systems which overcomes the limitations associated with the traditional syntheses of biologically active compounds comprised of thiazole and/or oxazole ring systems. Moreover, the present invention provides a large array of diverse compounds comprised of thiazole and/or oxazole ring systems which can be screened for biological activity, and as described below, are biologically active.

Summary of the Invention

Broadly this invention is directed toward a novel method for synthesizing an

ensemble of peptides that allows for the generation of an unlimited number of antibiotic compounds. The compounds synthesized find utility in inhibiting DNA replication or DNA transcription in cancer cells, pathogenic cells such as bacteria, and virally infected cells. The invention utilizes synthetic unnatural heterocyclic amino acids as building blocks in a solid phase combinatorial synthesis. More specifically, this invention is directed toward combining synthetic heterocyclic amino acids containing thiazole and/ or oxazole as building blocks in the synthesis of combinatorial libraries.

In a preferred embodiment of the invention, *N*-protected thiazole and/or oxazole containing amino acids are synthesized. These compounds are set forth below:



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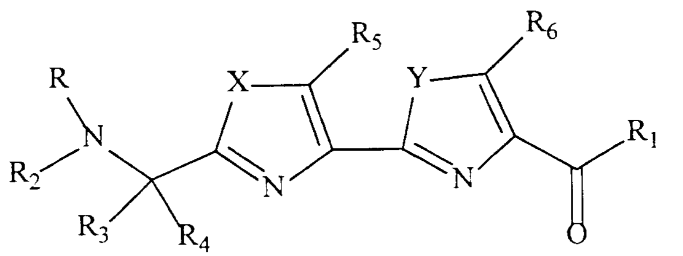


(12)

where R= H, a naturally occurring or synthetic **L** or **D** amino acid, *Tert*-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), carbobenzoxy (Z), Benzozyl (Bz), and other like amino protecting groups;

where R_1 = OH, alkyl esters, aromatic esters such as methyl, ethyl, *t*-butyl and benzyl, activated esters such as pentafluorophenyl, nitrophenyl, N-hydroxysuccinimide, acid chlorides, fluorides, organic salts, such as cyclohexyamines (CHA), amides, an amide bonded to a linker such as a diamine, or an insoluble support for use in solid phase synthesis;

where R_2 = H, a C_1-C_{10} alkyl or an aromatic ring;

where R_{3-4} = H, or a C_1-C_{10} alkyl;

where R_{5-6} = H, C_1-C_{10} alkyl, a heterocylic ring, an aliphatic or aromatic ring, a functional group such as an amine, an alchohol, a halide or an organometallic complex;

where X = oxygen (O) or sulfur (S);

where Y = oxygen (O) or sulfur (S);

The building blocks **11** and **12** are coupled with natural amino acids in a solid phase combinatorial synthesis to yield libraries of antibiotic compounds.

One aspect of the invention is the syntheses which form compounds **11** and **12**.

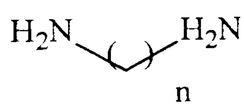
Another aspect of the invention is compound **12** where X= O and Y =S.

Another aspect of the invention is the coupling of compounds **11** and **12** with natural amino acids to yield naturally occurring antibiotic compounds.

Still another aspect of the invention are the antibiotic compounds that form the libraries.

Still another aspect of the invention are the syntheses which form the antibiotic compounds.

Still another embodiment of the invention is the solid phase combinatorial



synthesis where a distinct linker molecule having the structure:

where n= 1-10

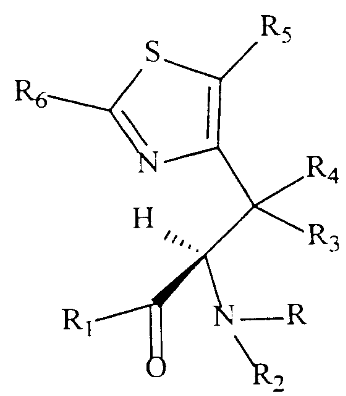
is used to attach a building block to a solid support.

Still another embodiment of the invention is the combination of the solid phase-linker-building block(s).

The advantages of the invention are that the synthesized building blocks, **11** and **12** have restricted conformations that are presented in synthetic packages (Fmoc or Boc) which facilitates their incorporation into standard peptide methodology. Another advantage of the invention is that the design of the synthesis for the building blocks is flexible enough to allow the preparation of any combination of oxazole and thiazole rings in a given two-ring building block, such as compound **12**, where X=O and Y=S, from naturally occurring amino acid starting materials. Furthermore, the peptide library can also incorporate any commercially available amino acid without the development of

new chemistry.

Another aspect of the invention embodies the libraries of antibiotic compounds formed by the coupling at least one of the following compounds:



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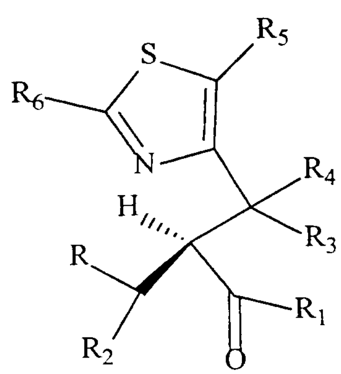


(15)

where R= H, a naturally occurring or synthetic **L** or **D** amino acid, *Tert*-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), carbobenzoxy (Z), Benzozyl (Bz), and other like amino protecting groups;

where R_1 = OH, alkyl esters, aromatic esters such as methyl, ethyl, *t*-butyl and benzyl, activated esters such as pentafluorophenyl, nitrophenyl, N-hydroxysuccinimide, acid chlorides, fluorides, organic salts, such as cyclohexyamines (CHA), amides, an amide bonded to a linker such as a diamine, or an insoluble support for use in solid phase synthesis;

where R_2 = H, a C_1-C_{10} alkyl, or an aromatic ring;

where R_{3-4} = H, or a C_1-C_{10} alkyl;

where R_{5-6} = H, C_1-C_{10} alkyl, a heterocylic ring, an aliphatic or aromatic ring, a functional group such as an amine, an alchohol, a halide or an organometallic complex;

with natural amino acids in a solid phase combinatorial synthesis to yield libraries of antibiotic compounds.

In the above structures the stereochemistry of the chiral R groups can independently be in the R or S configuration or a mixture of the two.

Brief Description of the Drawing(s)

Fig. 1 is a schematic showing one embodiment of the novel synthesis of compound 2-(Fmoc-aminomethyl)-thiazole-4-carboxylic acid.

Fig. 2 is a schematic showing one embodiment of the novel synthesis of 2-(Fmoc-aminomethyl)-oxazole-4-carboxlic acid.

Fig. 3 is a schematic showing one embodiment of the novel synthesis of 2-(2'-Fmoc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylic acid.

Fig. 4 is a graph showing the effects of **L2-6** and **L2-9** on the growth of marine bacterium *Vibrio angullarum*.

Fig. 5 is a graph showing the effect of a Microcin B17 fragment synthesized according to one embodiment of the invention on the growth of marine bacterium *Vibrio angullarum*.

Fig. 6 is a graph showing the effect of peptide control tachyplesin on the growth of marine bacterium *Vibrio angullarum*.

Description of the Preferred Embodiment(s)

Results and discussion

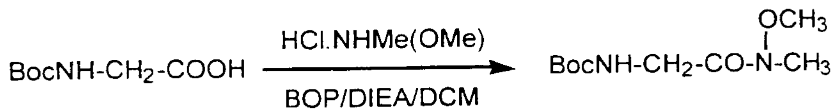
Synthesis of 2-(Fmoc-aminomethyl)-thiazole-4-carboxylic acid (**1**)

The preparation of compound **1** and R_{2-6}=H, using the Hantzsch synthesis has been reported. Referring to Fig. 1, the synthetic strategy disclosed herein is totally different from the reported one.

Cyclocondensation of the Boc-amino aldehyde prepared from its Boc-amino acid via the *N*-methoxy-*N*-methyl amide with L-cysteine methyl ester provided the thiazolidine, followed by dehydrogenation with active manganese dioxide to afford the thiazole product.

The coupling between Boc-glycine and *O,N*-dimethylhydroxylamine hydrochloride with benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium

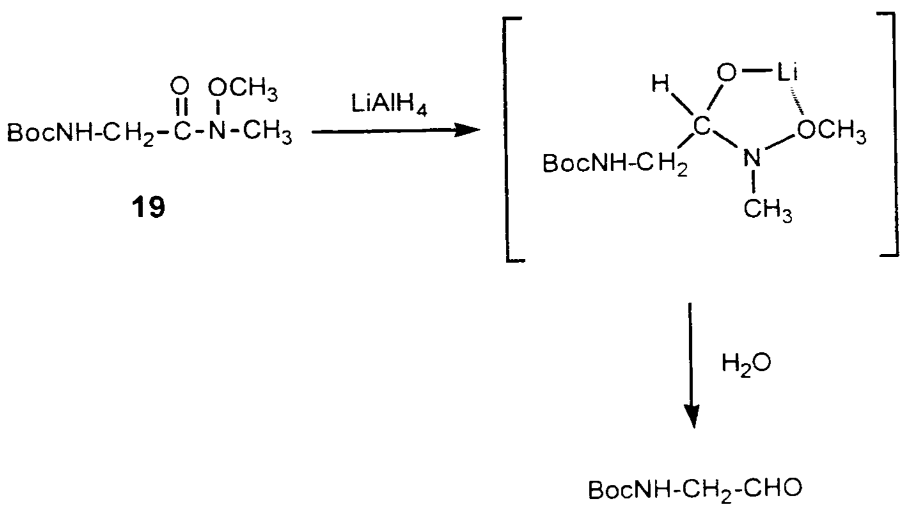
hexafluorophosphate (BOP) and with 10-min preactivation in the presence of *N*, *N*-diisopropylethylamine (DIEA) (31) at room temperature in 20 min. afforded the amide **19**. This reaction was fast and proceeded cleanly with a high yield (80%). The characteristic signals in the ^{1}H-NMR spectrum of **19** at δ 3.70 (s, 3 H, -HN-OCH_3), 3.18 (s, 3 H, -NH-CH_3), and 1.45 (s, 9 H, *t*-butyl-O-) confirmed the formation of **19**.



19

N-methoxy-*N*-methylamides are well known in the art as carbonyl equivalents in organic synthesis. The advantages of the use of this synthesis is the ease of preparation, and selective reduction to form the aldehydes. *N*-methoxy-*N*-methylamides can be prepared from the corresponding carboxylic acids and *N*, *O*-dimethylhydroxylamine with peptide coupling reagents such as BOP, DCC and *i*-butyl chloroformate.

The prepared Boc-Gly-*N*-methoxy-*N*-methylamide **19** in anhydrous THF was reduced with lithium aluminum anhydride in anhydrous diethyl ether for 30 min. at 0°C, followed by addition of a solution of potassium hydrogensulfate to afford **20** in high yield (89%). The ^{1}H-NMR spectrum showed the expected signal of the aldehyde proton at δ 9.60 (s, 1 H). In the product, a small amount of impurities were detectable on TLC (hexane-EtOAc = 1: 1).



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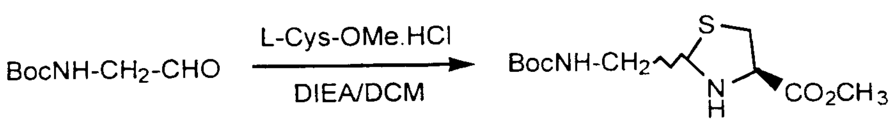
Reduction of **19** with lithium aluminum hydride gave a stable complex which prevented

further reduction to the alcohol. Upon hydrolysis of the complex, the expected aldehyde was formed. If the solubility of the *N*-methoxy-*N*-methylamide is low in ethyl ether, then the *N*-methoxy-*N*-methylamide can be reduced in anhydrous THF. When THF was used, it was found that a mixture of ether /THF achieved higher yield than did THF alone, and lower percentages of THF in ether gave higher yields.

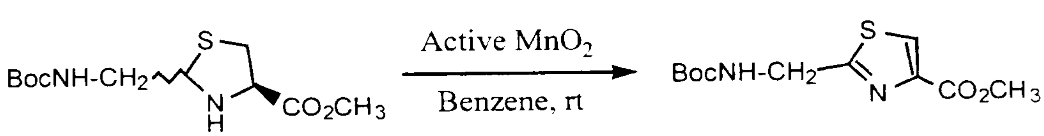
The cyclcondensation of Boc-glycinal **20** with L-cysteine methyl ester was achieved by dropwise addition of a solution of L-cysteine methyl ester hydrochloride and DIEA in methylene chloride to a solution of **20** in methylene chloride at room temperature. The reaction instantly afforded **21** (77%). The ^{1}H-NMR spectrum showed that **21** is a mixture of the two possible diastereomeric thiazolidines (ca. 50:50).



20 **21**

Unlike the conditions reported in the literature this condensation reaction was stirred overnight in benzene or in a slurry of magnesium sulfate in methylene chloride, it was found this reaction finished smoothly and instantly in methylene chloride. There is no need to prolong this reaction overnight or add magnesium sulfate to the reaction solution.

The dehydrogenation of 2-Boc-aminomethyl-thiazolidine-4-carboxylic methyl ester **21** was performed in benzene with manganese (IV) oxide (activated) at 55°C for 60 min to afford **22** (60%). The ^{1}H-NMR spectrum showed the expected signal of aromatic proton at δ 8.10 (s, 1 H). The UV spectrum displayed a maximum absorbance at 236 nm which is consistent with the reported data for thiazole rings.



21 **22**

Dehydrogenation on active manganese dioxide can proceed either by an ionic mechanism or a free radical mechanism. The precise elucidation of the mechanism is difficult because of the nature of the heterogeneous reaction involved. A large excess of active manganese dioxide (ca. 30 eq.) was required for the efficient dehydrogenation of

21. The purity of **21** played a critical role in the success or failure of this reaction. It was found that the oxidation of crude **21** by active manganese dioxide produced a complicated product mixture (dark solution and many spots on the TLC), resulting in a low yield (ca. 10%). With a large excess of active manganese dioxide and purified **22**, this reaction finished smoothly and cleanly within 60 min.

Alkaline hydrolysis of **22** in a THF/water solution (5:1) afforded 2-Boc-aminomethyl-thiazole-4-carboxylic acid **23** in high yield (92%).

The conversion of the Boc protecting group of **23** to the Fmoc protecting group was achieved by removal of Boc protecting group of **23** with TFA in methylene chloride (1:1), followed by reaction with Fmoc-OSu to re-protect the amino group of **23** with Fmoc to provide 2-(Fmoc-aminomethyl)-thiazole-4-carboxylic acid **1**. RP-HPLC analysis showed that the product has one peak. The ESI-MS measured molecular weight of **1** is consistent with the calculated mass.

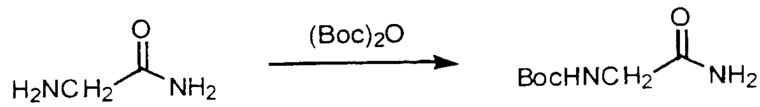
After the Boc deprotection of **23**, the residue was neutralized with sodium carbonate and used without purification in the next step. Protection of the free amino group from **23** with Fmoc-OSu was achieved by reacting the compounds in a THF/water (2:1) solution in the presence of sodium carbonate (1 eq.). Unlike the normal preparation of Fmoc-amino acids, an excess of Fmoc-OSu was used because the unusual amino acid is more expensive. It was hard to remove Fmoc-OSu from the product by recrystallization. Thus, after the reaction was finished, washing the reaction mixture with methylene chloride was a necessary and simple way to remove the excess reagent.

Synthesis of 2-(Fmoc-aminomethyl)-oxazole-4-carboxylic acid (2)

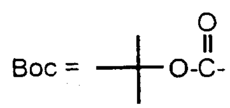
2-Fmoc-aminomethyl-oxazole-4-carboxylic acid (**2**), where R=Fmoc, R_1=OH, and $R_{2\text{-}6}$=H, was synthesized before by the imino ether method. Referring to Fig. 2., we used the same strategy as reported: cyclocondensation of Boc amino acid imino ether and L-serine methyl ester hydrochloride salt afforded the oxazoline, followed by dehydrogenation to produce the corresponding oxazole amino acid. The difference the method disclosed herein and the reported one is that triethyloxonium tetrafluoroborate replaced triethyloxonium hexafluoro-phosphate in the imino ether preparation step and the $CuBr_2$/DBU/HMTA reagent was used instead of the DBU/CCl_4/acetonotrile/pyridine reagent in the dehydrogenation step.

Boc-Glycine amide (**24**) was prepared according to the method reported in Stewart et al., *Solid Phase Peptide Synthesis*, 2nd ed., 63, Pierce Chemical Compnay,

Illinois, (1984), which is hereby incorporated by reference in its entirety into this disclosure. Di-*t*-butyl dicarbonate was added dropwise to a solution of glycine amide hydrochloride and one equivalent of sodium hydroxide in a water/*t*-butanol mixture (1:2) over a period of 15 min. After 15 min, more *t*-butanol was added to the reaction solution. The reaction was smooth and fast, finishing within one hour (yield 82%). The ^{1}H-NMR spectrum showed the signal of Boc at δ 1.46 (s, 9 H, *t*-butyl-O-), and confirmed the formation of **24**.

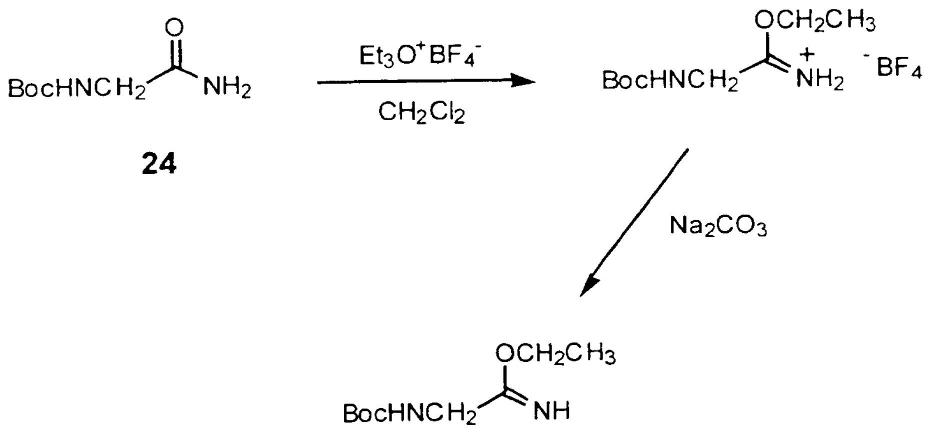


24



Amide **24** can easily dissolve in either methylene chloride or water. Therefore, after the reaction is finished and the *t*-butanol is removed, the volume of the residual aqueous mixture should be kept to a minimum. Otherwise, organic solvents such as ethyl acetate were unable to efficiently extract **24** from large volumes of aqueous mixtures for the purpose of purification. We found that DCM-benzene was a good system for recrystallizing amide **24**.

To prepare Boc-aminoacetimino ethyl ether (**25**), Boc-glycine amide (**24**) was dissolved in a large volume of methylene chloride under argon, and was treated with triethyloxonium tetrafluoroborate for six hours at room temperature. Then, the reaction solution was diluted with more methylene chloride and the mixture was neutralized by pouring it into an icy sodium bicarbonate solution to afford **25**. The ^{1}H-NMR spectrum displayed the signals of an ethyl ether at δ 4.16 (q, 2 H, J = 7.0 Hz, -O-$\mathbf{CH_2}$-CH_3) and 1.29 (t, 3 H, J = 7.0 Hz, -O-CH_2-$\mathbf{CH_3}$), and the Boc at 1.46 (s, 9 H, *t*-butyl-O-), consistent with the structure of Boc-aminoacetimino ethyl ether **25**.



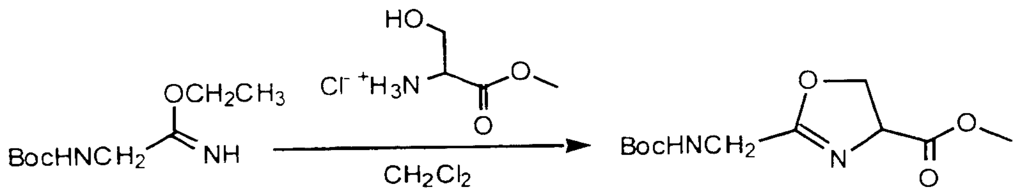
24

25

Triethyloxonium tetrafluoroborate is a powerful ethylating agent. It was reported that treatment of the amide with one equivalent of triethyloxonium tetrafluoroborate in methylene chloride at room temperature gave the imino ether. In addition, one equivalent of tetrafluoroboric acid (HBF_4) was generated during the reaction, which was considered a potential problem, because Boc protecting group is removed in strong acids. This reaction was performed in a large volume of solvent to dilute the acid generated in situ and the reaction was stopped after six hours even though there was trace of starting material remaining. Prolonging the reaction time was demonstrated to be detrimental to the product yield. Commercially available triethyloxonium tetrafluoroborate solution in methylene chloride (1M) in this reaction destroyed the starting material quickly and completely.

Boc-aminoacetimino ethyl ether **25** can not be purified by silica gel column chromatography, as it completely decomposed on the column. Thus, **25** was used without further purification.

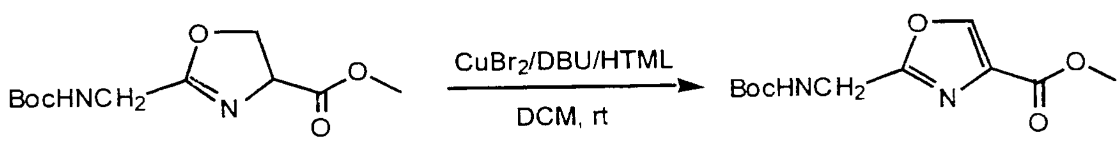
After **25** was prepared, it was immediately reacted with L-serine methyl ester hydrochloride in methylene chloride at room temperature for 24 hours to afford methyl 2-(Boc-aminomethyl)-oxazoline-4-carboxylate (**26**). The ^{1}H-NMR spectrum showed the signals of methoxyl at δ 3.76 (s, 3 H, $-OCH_3$) and Boc at 1.43 (s, 9 H, *t*-butyl-O-).



25 **26**

Oxazoline **26** is not stable in organic solvents or exposure to the air when dry. It is known in the art that when a pure oxazoline-containing amino acid methyl ester is exposed to the air for a couple of weeks, the oxazoline ring was open to form the corresponding dipeptide.

Dehydrogenation of **26** was achieved by treatment with four equivalents of $CuBr_2$/DBU/HTMA in methylene chloride at room temperature, and after 10 hours the reaction mixture was recharged with the reagent to react another day. After purification of the reaction mixture by partition and by silica gel column chromatography (hexane-EtOAc = 4:1, 3:1 and 2:1), methyl 2-(Boc-aminomethyl)-oxazole-4-carboxylate (**27**) was obtained. The ^{1}H-NMR spectrum of **27** showed the aromatic proton peak of at δ 8.17 (s, 1 H), which confirmed the formation of an oxazole ring. The UV spectrum showed a maximum absorbance at 210 nm.



26 **27**

The dehydrogenation of a small quantity of **26** was satisfactorily achieved by the use of a $CuBr_2$/DBU/HMTA reagent.

2-Boc-aminomethyl-oxazole-4-carboxylic acid (**28**) was obtained from **27** by alkaline hydrolysis of **27** in THF/water solution for 2 hours in high yield (91%). The ^{1}H-NMR spectrum showed the signals of the oxazole aromatic proton at δ 8.39 (s, 1 H), and Boc at 1.44 (s, 9H, *t*-butyl-O-). The disappearance of methoxyl signal from the ^{1}H-NMR spectrum confirmed that the hydrolysis of **27** was complete.

The Boc protecting group of **28** was removed smoothly by TFA-DCM (1:1) in 45 min at room temperature.

After removal of Boc protecting group, without further purification, the residue

was neutralized to re-protect the amino group by treatment with an excess of Fmoc-OSu and two equivalents of sodium carbonate in THF/water solution (2:1) to afford Fmoc-protected amino acid **2** (85%). The ^{1}H-NMR spectrum of the product showed the signals of Fmoc aromatic protons at δ 7.77-7.17 (m, 8H), confirmed the presence of Fmoc in **2**. RP-HPLC analysis showed that the product has one peak. The ESI-MS measured molecular weight of **2** is consistent with the calculated mass.

<u>Synthesis of 2-(2'-Fmoc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylic acid **3**</u>

The synthesis of 2-(2'-Fmoc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylic acid **3** has been reported in Videnov et al., *Angew. Chem. Int. Ed. Engl.* **35** (13/14), 1503 (1996).

Referring to Fig. 3, a different strategy is disclosed herein: cyclocondensation of protected L-serinal (from protected L-serine) via its *N*-methoxyl-*N*-methyl amide with L-cysteine methyl ester afforded protected (Ser)-thiazolidine methyl ester, followed by dehydrogenation to give protected (Ser)-thiazole methyl ester. Then, protected (Ser)-thiazole methyl ester was deprotected and condensed with Boc-glycine imino ether to form the Boc-oxazolinyl thiazole which was dehydrogenated to afford the Boc-oxazolyl thiazole amino acid product.

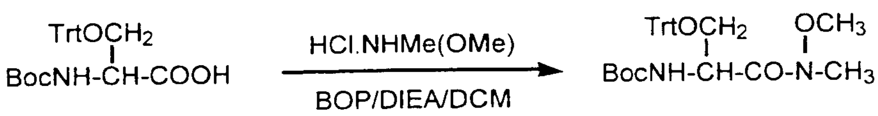
The advantage of this strategy is that the first thiazole intermediate is easier to synthesize, and is converted to the oxazolyl thiazole in two steps which minimized the loss of this intermediate.

Boc-Ser(Trt)-OH (**29**) was obtained by removing the Fmoc of commercially available Fmoc-Ser(Trt)-OH in diethylamine-methylene chloride (3:4) and then re-protecting the amino group of Ser(Trt)-OH with di-*t*-butyl dicarbonate in *t*-butanol aqueous solution (yield 78%). The ^{1}H-NMR spectrum of **29** showed the signals of Boc at δ 1.44 (s, 9 H, *t*-butyl-O-), trityl at 7.5-7.1 (m, 15 H) and serine at 10.86 (s, br, 1 H, -COOH), 5.35 (d, 1 H, J = 8.3 Hz), 4.41 (m, 1 H), 3.61 (dd, 1 H, J = 9.1 and 3.3 Hz), 3.38 (dd, 1 H, J = 9.1 and 3.4 Hz), which confirmed the formation of **29**.

The reason for converting Fmoc-Ser(Trt)-OH to Boc-Ser(Trt)-OH was to make the protecting groups compatible so that the protecting groups Boc and Trt could be removed simultaneously with TFA in later synthesis. Contrary to Boc, Fmoc could be removed by bases.

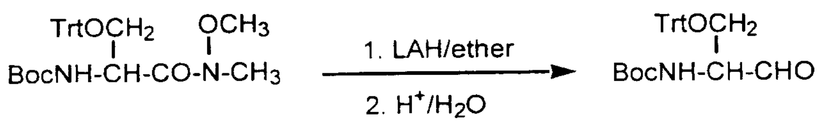
Boc-Ser(Trt)-OH (**29**) was then coupled with *O*,*N*-dimethylhydroxyl-amine

hydrochloride under BOP activation in the presence of DIEA in methylene chloride for 20 min. to afford Boc-Ser(Trt)-*N*-methoxy-*N*-methyl amide (**30**). After purification by silica gel column chromatography (solvent system: hexane-EtOAc = 5:1 and 3:1), **30** was obtained in high yield (98%). The ^{1}H-NMR spectrum of **30** showed the characteristic signals of the trityl group at δ 7.5-7.1 (m, 15 H), *N*-methoxy group at 3.57 (s, 3 H), *N*-methyl group at 3.18 (s, 3 H), and Boc at 1.43 (s, 9 H).



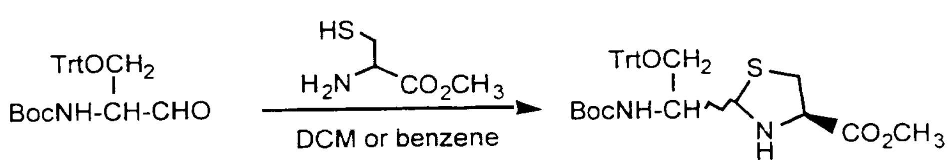
29 **30**

N$^{\alpha}$-Boc-O-trityl-L-serinal (**31**) was prepared from **30** by the reduction of **30** with lithium aluminum hydride in anhydrous ethyl ether under argon at 0°C for 30 min., followed by hydrolysis with aqueous potassium hydrogensulfate solution to produce **31** (yield 94%). The ^{1}H-NMR spectrum showed the aldehyde signal at δ 9.52 (s, 1 H, -CHO), which confirmed the formation of an aldehyde.



30 **31**

Without purification, **31** was used to condense with L-cysteine methyl ester in methylene chloride at room temperature, then in benzene to afford **32** (yield 95%). The ^{1}H-NMR spectrum showed the signals of trityl protons at δ 7.5-7.1 (m, 15 H, trityl), methoxyl protons at 3.72 (s, 3 H, $-OCH_3$), and Boc at 1.45 (s, 9 H, *t*-butyl-O-).

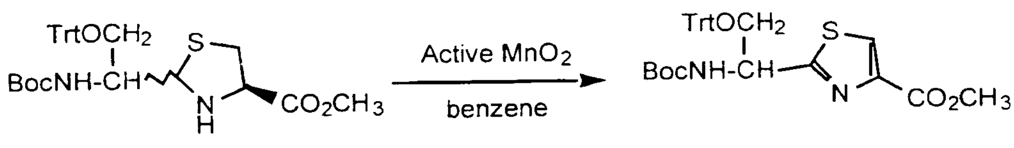


31 **32**

In case, **31** absorbed water from the air to form the undesired aldehyde hydrate, benzene was added to remove any water so that the equilibrium was shifted to force the cyclocondensation reaction to completion.

Thiazolidine **32** was dehydrogenated by active manganese dioxide in benzene at

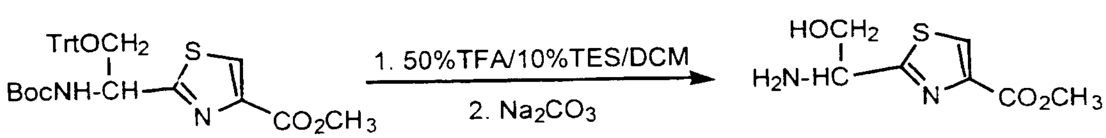
50°C for five hours to afford thiazole **33** (yield 59%). The [1]H-NMR spectrum and 2-D COSY experiment showed the signals of **33** at δ 8.06 (s, 1 H, aromatic), 7.5-7.1 (m, 15 H, trityl), 5.61 (d, 1 H, J = 7.7 Hz), 5.17 (m, 1 H, α-H), 3.88 (s, 3 H, $-O-CH_3$), 3.78 (dd, 1 H, J = 4.1 and 9.1 Hz,β-H), 3.46 (dd, 1 H, J = 9.2 and 4.1 Hz, β-H'), and 1.42 (s, 9 H, *t*-butyl-O), confirming the formation of **33**.



32 **33**

TLC monitoring of the dehydrogenation showed that one of the two thiazolidine diastereomers was quickly oxidized to the product, while the other was very resistant to oxidation. The resistant isomer needed a longer reaction time, and maybe additional oxidant for an efficient oxidation. Overall, longer reaction times should be avoided because the Boc protecting group is slightly thermo-labile.

After the protected (Ser)-thiazole methyl ester **33** was synthesized, both protecting groups (trityl and Boc) of **33** were removed in TFA/triethylsilane/DCM (50: 10: 40 v/v/v) for 45 min at room temperature. After purification, **34** was obtained (yield 90%). The [1]H-NMR spectrum showed the signals of an aromatic proton at δ 8.26 (s, 1H), α-H at 4.26 (dd, 1H, J = 4.8 and 6.2 Hz.), methoxy group at 3.88 (s, 3H), β-H at 3.86 (dd, 1H, J = 10.7 and 4.8 HZ), and β-H' at 3.69 (dd, 1H, J = 10.7 and 6.2 HZ), which confirmed the formation of **34**.



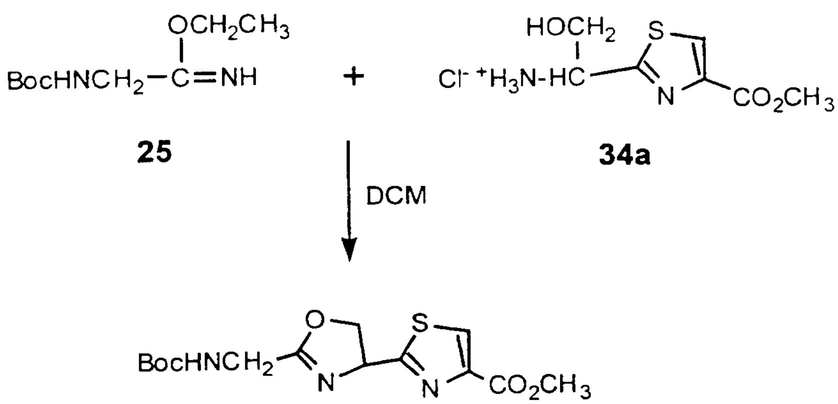
33 **34**

When deprotecting the Boc and trityl groups of **33**, triethylsilane was added as a scavenger for the stable trityl cation so that the deprotection could proceed to completion. During purification, although triphenyl methane was washed away by methylene chloride, there was still a large quantity of water soluble impurity in the residue. It is simple and efficient to use a Sephadex® LH-20 column (eluent: methanol) to purify the residue.

To prepare the hydrochloride salt **34a** of **34**, **34** was dissolved in 1 M

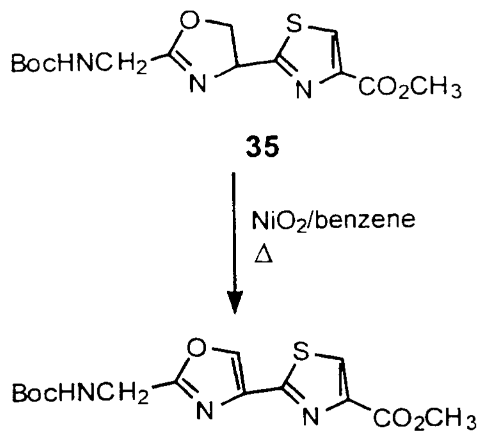
hydrochloric acid (aqueous solution), and concentrated to dryness *in vacuo* at room temperature.

The hydrochloride salt **34a** was added to a solution of Boc-aminoacetimino ethyl ether **25** in methylene chloride and reacted for 24 hours at room temperature. After purification, **35** was obtained (yield 44%). The ^{1}H-NMR spectrum of **35** showed signals at δ 8.10 (s, 1H, aromatic), 5.53 (dd, 1H, J = 7.9 and 9.6 Hz, oxazoline-H-4), 5.17 (s, br, 1H), 4.85-4.40 (m, 2H, oxazoline-H-5), 4.03 (d, 2H, J = 5.7 Hz), 3.93 (s, 3H, -O-CH_3), and 1.46 (s, 9H, *t*-butyl-O-), which confirmed the formation of **35**.



35

Methyl 2-(2'-Boc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylate (**36**) was obtained by dehydrogenation of **35** with nickel peroxide in benzene at 70°C (yield 22%). The ^{1}H-NMR spectrum showed the signals of the thiazole and oxazole aromatic protons at δ 8.28 (s, 1H) and 8.15 (s, 1H), methoxy group at 3.95 (s, 3H), and Boc at 1.47 (s, 9H).



35

36

Dehydrogenation of oxazolines can be achieved by nickel peroxide in benzene by reflux for several hours to several days as reported in Evans et al. *J.Org. Chem.*, **44** (4), 497 (1979) and Knight et al., *Synlett*, **1**, 36 (1990), which are hereby incorporated by reference in their entireties into this disclosure.

The dehydrogenation of **36** by nickel peroxide proceeded smoothly. It was not necessary to reflux benzene solution for a long time. TLC monitoring (hexane-acetone = 1:1) showed that the reaction finished within two hours at 70°C. The reaction had a low yield. For a fast and complete dehydrogenation, at least three equivalents of active oxygen from nickel peroxide is required. Prolonged reaction times should be avoided because the Boc protecting group is labile to heating, which will result in a low reaction yield. Also, active oxygen was lost by heating nickel peroxide for a long time. Therefore, if the reaction is not complete within two hours, a second or third charge of nickel peroxide is recommended.

Alkaline hydrolysis of **36** in THF/water (4:1) solution for two hours at room temperature afforded **37** (yield 90%). The ^{1}H-NMR spectrum showed the signals of the thiazole and oxazole protons at δ 8.46 (s, 1 H) and 8.34 (s, 1H), the methylene group at position 2 of the oxazole at 4.40 (s, 2H), and the Boc at 1.47 (s, 9H, *t*-butyl-O-).

The last step of the synthesis involved converting the amino protecting group of **37** from Boc to Fmoc. **37** was treated with 40%TFA in DCM for 60 min to completely remove the Boc protecting group. The residue from the deprotection of **37** was neutralized and was directly treated with Fmoc-OSu in a THF/water (2:1) solution in the presence of sodium carbonate for two hours at room temperature to give Fmoc-protected amino acid **3** (yield 77%). The ^{1}H-NMR spectrum of **3** showed the signals of the

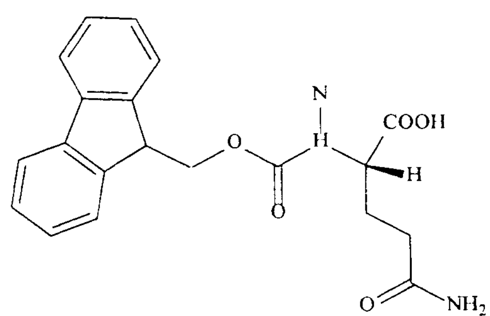
thiazole and oxazole protons at δ 8.74 (s, 1H) and 8.38 (s, 1H), and the aromatic protons of Fmoc at 7.89-7.31 (m, 8H). RP-HPLC analysis showed that the product has one peak. The ESI-MS measured molecular weight of **3** is consistent with the calculated mass.

Compound **3** did not dissolve in DCM, ethyl ether, EtOAc, THF, methanol, ethanol, acetonitrile, HFIP, DMF, NMP or water. It could only dissolve in DMSO, or the solutions containing at least 50% DMSO in DMF or NMP.

Synthesis of Fmoc-glutamine (38)

The N-α protection of L-glutamine with Fmoc was achieved by treatment with Fmoc-OSu in THF/water (2:1) solution in the presence of sodium carbonate overnight at room temperature to afford **38** (84%). The 1H-NMR spectrum of **38** showed the signals of the Fmoc aromatic protons at δ 7.89-7.20.

In this reaction, an excess of L-glutamine was used so that the purification of **38** was easier. After the reaction was finished, the reaction mixture was diluted with acidic aqueous solution. By filtration, the solid product **38** was collected and the excessive L-glutamine in solution was removed.



(38)

Experimental Section

General

Lithium aluminum hydride (LAH), *N*, *O*-dimethylhydroxylamine hydrochloride, tetrahydrofuran (anhydrous) (THF), potassium hydrogensulfate, L-cysteine methyl ester hydrochloride, diethylamine (DEA), and triethylsilane (TES) were purchased from Aldrich. *N*, *N'*-Dicyclohexylcarbodiimide (DCC), manganese (IV) oxide (activated), hexamethylenetetramine (HMTA), *N*, *N*-diisopropylethylamine (DIEA), nickel peroxide, 1,8-diazabicyclo [5.4.0] undec-7-ene (1,5-5) (DBU), triethyloxonium tetrafluoroborate, and cupric bromide were purchased from Fluka. Boc-glycine, glycine amide

hydrochloride, benzotriazole-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP), trifluoroacetic acid (TFA), *N*-(9-Fluorenylmethyloxycarbonyl) oxysuccinimide (Fmoc-OSu), di-*t*-butyl dicarbonate, and L-serine methyl ester hydrochloride were from Advanced ChemTech. Dimethyl sulfoxide (DMSO), dichloromethane (DCM), and *N*, *N*-dimethylformamide (DMF) were from Burdick & Jackson. *t*-Butanol, L-glutamine, hexane, acetonitrile and diethyl ether (anhydrous) were from Fisher. Fmoc-Ser(Trt)-OH and benzotriazole-1-yl-oxy-tripyrrolidino-phosphonium hexafluorophosphate (PyBOP) were from Novabiochem.

Silica gel 60 for column chromatography (70-230 mesh) and silica gel TLC plates F254 (plastic or aluminum-backed sheet) were from E. Merck.

TLC developing solvent systems: (1) hexane-EtOAc; (2) hexane-acetone; (3) chloroform-MeOH-glacial HOAc (100:5:2 or 100:10:4). Methods for TLC visualization: 1. Examine the plate under UV light (254 nm); 2. Expose the plate to I_2 vapor in a jar for 5 min; 3. Spray the plate with a solution of 0.2% ninhydrin in 95% ethanol - 10% aqueous acetic acid (95:5) and then heat at 110°C for 5 min.

HPLC analysis was performed on a Waters HPLC system using a Vydac 218TP C18 10 □m reversed-phased column (250 x 4.6 mm) with a mobile-phase gradient: 40%-70% acetonitrile in 0.1% (v/v) TFA over 30 min, flow rate 1.0 ml/min, and UV detection at 215 and 290 nm. 18.2 MΩ water was produced by a Millipore Milli-Q plus system (Millipore, Bedford, MA).

UV spectra were recorded on a Hitachi U-2000 spectrophotometer.

ESI-MS were measured at Pfizer Central Research (Groton, CT) on a PE SCIEX API-100B LC/MS System (Foster City, CA). Mode: ESI, single quad, m/z = 300-2200, 4.2 sec/scan, flow rate: 200 μl/min, acetonitrile-water (50:50) in 0.1% TFA (v/v), and processed using BioMultiview 1.3 alpha program.

The ^1H NMR spectra were obtained on a modified EM-390 Varian spectrometer (EFT-90-30, Anasazi Instruments Inc., Indianapolis, IN), and processed with NUTS program (Win95 version, Acorn NMR Inc., Fremont, CA). Chemical shifts (δ) are given in ppm downfield from tetramethylsilane (TMS). Abbreviations for peak description are s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad.

<u>Synthesis of 2-(Fmoc-aminomethyl)-thiazole-4-carboxylic acid (**1**)</u>

<u>Boc-Gly-*N*-methoxy-*N*-methyl amide (**19**)</u>

To a well stirred solution of Boc-Gly (21.02 g, 0.12 mole) and BOP (53.10 g,

0.12 mole) in 300 ml of DCM, was added DIEA (20.88 ml, 0.12 mole). After 10 min, a solution of *O,N*-dimethylhydroxylamine hydrochloride (14.05 g, 0.144 mole) and DIEA (31.32 ml, 0.18 mole) in 100 ml of DCM was added to above stirred solution. The reaction was monitored by TLC (silica gel, hexane-EtOAc = 2:1). After 20 min, 200 ml of DCM was added to the reaction solution. The DCM solution was washed successively with 1 N aqueous hydrochloric acid solution (500 ml x 4), saturated aqueous sodium bicarbonate solution (500 ml x 3), and saturated aqueous sodium chloride solution (500 ml). The organic solution was dried with 5 g of magnesium sulfate overnight, filtered, and concentrated under reduced pressure. The residue was dissolved in a minimal volume of DCM, followed by addition of hexane until the solution became cloudy. The solution was warmed until it became clear and then kept to stay at room temperature to give colorless needles of **19** (21 g). Yield: 80%. TLC R_f = 0.24 (hexane-EtOAc = 2:1).^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 5.21 (s, br, 1 H), 4.05 (d, 2 H, J = 5.0 Hz), 3.70 (s, 3 H), 3.18 (s, 3 H), and 1.45 (s, 9 H).

Boc-glycinal (**20**)

Boc-Gly-*N*-methoxy-*N*-methylamide (**19**) (4.37 g, 20 mmole) in 150 ml of anhydrous THF was stirred in a ice-water bath under argon for 30 min. A solution of LAH in diethyl ether (1 M) (30 ml, 30 mmole) was added to the above well stirred solution by cannula under argon. The resulting solution was stirred for 30 min. A solution of potassium hydrogensulfate (4.77 g, 35 mmole) in 60 ml of water was gradually added to the reaction solution and stirred for 10 min. Organic solvents in the reaction mixture were evaporated under reduced pressure. An additional 60 ml of water was added to the aqueous residue, which was then extracted with DCM (100 ml x 4). The combined DCM extracts were washed with 1 M hydrochloric acid solution (100 ml x 4), saturated sodium bicarbonate solution (100 ml x 2), and saturated sodium chloride solution (100 ml), dried with 4 g of magnesium sulfate overnight, and filtered. Evaporation of the solvent under reduced pressure left a yellowish oil **20** (2.83 g) which was used without further purification. Yield: 89%. TLC R_f = 0.44 (hexane-EtOAc = 1:1). ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 9.60 (s, 1 H), 5.26 (s, br, 1 H), 4.04 (d, 2 H, J = 5.1 Hz), and 1.46 (s, 9 H).

Methyl 2-Boc-aminomethyl-thiazolidine-4-carboxylate (**21**)

To a stirred solution of Boc-glycinal (**20**) (2.83 g, 17.8 mmole) in 80 ml of DCM, was added (dropwise) a solution of L-Cys-OMe hydrochloride (3.86 g, 20 mmole) and

DIEA (6.0 ml, 34 mmole) in 50 ml of DCM. The reaction finished within 5 min. Evaporation of the reaction mixture under reduced pressure afforded a residue. The residue was purified by silica gel column chromatography (50 x 2 cm, solvent system: hexane-EtOAc = 2:1) to furnish 3.81 g of **21**. Yield: 77%. TLC R_f = 0.39 (hexane-EtOAc = 1: 1). ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 4.97 (s, br, 1 H), 4.9-4.6 (m, 1 H), 4.0-3.7 (m, 2 H), 3.76 (s, 3 H),3.4-2.7 (m, 3 H), 1.44 (s) and 1.43 (s) (9 H), which showed that **21** is a diastereomeric mixture (ca. 1:1).

Methyl 2-Boc-aminomethyl-thiazole-4-carboxylate (**22**)

A solution of 2-Boc-aminomethyl-thiazolidine-4-carboxylic methyl ester (**21**) (3.81 g, 13.8 mmole) in 150 ml of benzene was heated to 55°C. To above stirred solution, was added manganese (IV) oxide (activated) (30 g, 25 eq) and pyridine (1.5 ml). The reaction solution was stirred at 55°C for 60 min. After initial filtration, the insoluble material was washed with DCM (100 ml x 2). The combined filtrates were concentrated under reduced pressure and the residue was dissolved in minimal amount of DCM. Addition of hexane caused the DCM solution to become cloudy. The solution was warmed until it became clear and then kept at room temperature to give colorless needles of **22** (2.25 g). Yield: 60%. TLC R_f = 0.12 (hexane-acetone = 5:1). ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 8.10 (s, 1 H), 5.21 (s, br, 1 H), 4.63 (d, 2 H, J = 6.4 Hz), 3.94 (s, 3 H), and 1.47 (s, 9 H). UV: λ_{max} (MeOH) 236 nm (ε 5880 M^{-1} cm^{-1}).

2-Boc-aminomethyl-thiazole-4-carboxylic acid (**23**)

To a stirred solution of methyl 2-Boc-aminomethyl-thiazole-4-carboxylate (**22**) (2.53 g, 9.2 mmole) in 80 ml of THF, was added 20 ml of 1 N sodium hydroxide aqueous solution. After 60 min, the spot corresponding to starting material disappeared on silica gel TLC (solvent system: hexane-acetone = 1:1). The reaction solution was diluted with 200 ml of water and washed with DCM (300 ml x 2). Acidification of the aqueous layer with 10% potassium hydrogensulfate to pH 3 was followed by extraction into EtOAc (200 ml x 3). Concentration under reduced pressure of the dried EtOAc solution (magnesium sulfate) left a residue, which was recrystallized from MeOH-EtOAc-hexane to give a white powder **23** (2.21 g). Yield: 92%. TLC R_f = 0.10 ($CHCl_3$-MeOH-HOAc = 100:5:2). ^{1}H-NMR (90 MHz, DMSO-d_6) δ ppm: 8.28 (s, 1 H), 7.73 (s, br, 1 H), 4.37 (d, 2 H, J = 5.8 Hz), and 1.41 (s, 9 H).

2-(Fmoc-aminomethyl)-thiazole-4-carboxylic acid (**1**)

2-Boc-aminomethyl-thiazole-4-carboxylic acid (**23**) (2.21 g, 8.55 mmole) was dissolved in 120 ml of DCM-TFA (1:1) and stirred for 30 min. Removal of the solvent under reduced pressure was followed by neutralization of the residue with a solution of sodium carbonate (2 g, 19 mmole) in 40 ml of water, which was adjusted to pH 8 with additional solid sodium bicarbonate. Fmoc-OSu (4 g, 12 mmole) in 80 ml of THF was added to the resulting solution , and the mixture was stirred for 24 hours. The reaction mixture was concentrated under reduced pressure to remove THF and the residual liquid was washed with DCM (50 ml x 4), and acidified to pH 3 with 1 N hydrochloric acid solution. The precipitate formed in the solution was collected by filtration, dried *in vacuo*, and recrystallized from DMF-0.1 N HCl (aqueous solution) to afford colorless needles (**1**, 3.13 g). Yield: 96%. TLC R_f = 0.44 (CHCl3-MeOH-HOAc = 50:5:2). RP-HPLC anlysis: retention time = 10.45 min (average of two runs). ^{1}H-NMR (90 MHz, DMSO-d_6) δ ppm: 12.84 (s, br, 1H0, 8.30 (s, 1H), 7.89-7.17 (m, 8H), 5.15 (s, br, 1H), and 4.49-4.13 (m, 5H). ESI-MS (m/z): 381.3 $[M + H]^+$, calculated monoisotopic mass 381.09.

Synthesis of 2-Fmoc-aminomethyl-oxazole-4-carboxylic acid (**2**)

Boc-glycine amide (**24**)

To a stirred solution of glycine amide hydrochloride (11.06 g, 0.1 mole) and sodium hydroxide (4.0 g, 0.1 mole) in 25 ml of water and 50 ml of *t*-butanol, was added di-*t*-butyl dicarbonate (25 g, 0.11 mole) dropwise over a period of 15 min. After 15 min, an additional 50 ml of *t*-butanol was added to the reaction solution. The reaction was not stopped until the starting material disappeared, monitored by TLC (hexane-acetone = 1:1) (ca. one hour). Then, the organic solvent was removed under reduced pressure and the residual solution was diluted with 50 ml of water. The aqueous solution was washed with petroleum ether (200 ml x 3), acidified to pH 2 with 1 N hydrochloric acid solution, and extracted with EtOAc (200 ml x 5). The dried EtOAc solution (magnesium sulfate) was filtered, and concentrated under reduced pressure to leave a residue, which was recrystallized from DCM-benzene to give colorless powders **24** (14.3 g). Yield: 82%. TLC R_f = 0.36 (hexane-acetone = 1:1). ^{1}H-NMR (90 MHz, $CDCl_3$) δppm: 6.00 (s, br), 5.62 (s, br), 5.16 (s, br), 3.80 (d, 2 H, J = 5.8 Hz), and 1.46 (s, 9 H).

Boc-aminoacetimino ethyl ether (**25**)

To a stirred solution of Boc-glycine amide (**24**) (10.45 g, 60 mmole) in 600 ml of DCM under argon, was added triethyloxonium tetrafluoroborate (13.68 g, 95%, 68

mmole). The reaction solution was stirred under argon at room temperature for 6 hours and diluted with 400 ml of DCM, which was then poured into ice-cold sodium bicarbonate solution (1 M, 300 ml) and shaken well. The DCM layer was separated, dried over magnesium sulfate overnight, filtered, and concentrated under reduced pressure to afford an oily residue. This residue (crude **25**) was used in next step without further purification. TLC R_f = 0.23 (hexane-acetone = 4:1). ^{1}H-NMR (90 MHz, $CDCl_3$) δppm: 5.03 (s, br, 1 H), 4.16 (q, 2 H, J = 7.0 Hz), 3.72 (d, 2 H, J = 6.3 Hz), 1.46 (s, 9 H), and 1.29 (t, 3 H, J = 7.0 Hz).

Methyl 2-(Boc-aminomethyl)-oxazoline-4-carboxylate (**26**)

To a stirred solution of all Boc-aminoacetimino ethyl ether (**25**) from the previous step in 200 ml of DCM, was added L-serine methyl ester hydrochloride (8.4 g, 54 mmole). After being stirred for 24 hours at room temperature, the reaction mixture was concentrated under reduced pressure to leave a residue. The crude product was used in next step without further purification. TLC Rf = 0.46 (hexane-acetone = 1:1). ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 5.50 (s, br, 1 H), 4.9-4.4 (m, 3 H), 3.97 (d, 2 H, J = 5.6 Hz), 3.76 (s, 3 H), and 1.43 (s, 9 H).

Methyl 2-Boc-aminomethyl-oxazole-4-carboxylate (**27**)

To a stirred suspension of cupric bromide (26.8 g, 0.12 mole) in 750 ml of DCM, were added hexamethylenetetramine (HMTA) (16.82 g, 0.12 mole) and 1,8-diazabicyclo[5.4.0]undec-7-ene (1,5-5) (DBU) (18 ml, 0.12 mole). After the resulting brown solution stirred for 10 min at room temperature, the crude methyl 2-Boc-aminomethyl-oxazoline-4-carboxylate (**26**) from previous step was added. After 10 hours, the reaction vessel was recharged with 80 mmole each of cupric bromide, HMTA, and DBU, and stirred for another day. The mixture was filtered and the filtrate was concentrated under reduced pressure to afford a residue, which was partitioned between 600 ml of EtOAc and 600 ml of saturated aqueous NH_4Cl-concentrated NH_4OH (1:1). The aqueous layer was then extracted with EtOAc (200 ml x 3). The combined EtOAc extracts were washed with saturated aqueous NH_4Cl-concentrated NH_4OH (1:1) (150 ml x 4), 1 M hydrochloric acid solution (300 ml x 4), saturated sodium bicarbonate solution (300 ml), and saturated sodium chloride solution (300 ml), and dried by magnesium sulfate overnight. The dry EtOAc solution was filtered, and concentrated under reduced pressure to leave a residue, which was purified by silica gel column chromatography (10 x 4 cm, hexane-EtOAc = 4:1, 3:1 and 2:1) to give **27** (1.34 g). TLC R_f = 0.52 (hexane-

acetone = 1:1). ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 8.17 (s, 1 H), 5.50 (s, br, 1 H), 4.47 (d, 2 H, J = 5.8 Hz), 3.89 (s, 3 H), and 1.44 (s, 3 H). UV: λ_{max} (MeOH) 210 nm (ε 7000 M^{-1} cm^{-1}).

2-Boc-aminomethyl-oxazole-4-carboxylic acid (**28**)

A solution of methyl 2-Boc-aminomethyl-oxazole-4-carboxylate (**27**) (1.42 g, 5.5 mmole) in 80 ml of THF and 20 ml of 1 M sodium hydroxide (aqueous solution) was stirred for 2 hours. The solution was concentrated under reduced pressure to remove THF. The residual solution was diluted with 100 ml of water, washed with DCM (50 ml x 3), acidified to pH 2 by addition of 10% potassium hydrogensulfate (aqueous solution), and extracted with EtOAc (100 ml x 5). The EtOAc solution was dried over magnesium sulfate overnight. The dried EtOAc solution was filtered, and concentrated under reduced pressure to give powders **28** (1.21 g). Yield: 91%. ^{1}H-NMR (90 MHz, CD_3OD) δ ppm: 8.39 (s, 1 H), 4.36 (s, 2H), and 1.44 (s, 9H).

2-(Fmoc-aminomethyl)-oxazole-4-carboxylic acid (**2**)

A solution of 2-Boc-aminomethyl-oxazole-4-carboxylic acid (**28**) in 50 ml of TFA-DCM (1:1) was stirred for 45 min and concentrated under reduced pressure to dry. Water 10 ml was added to the residue, which was neutralized to pH 7 by adding 1 M sodium hydroxide (aqueous solution), followed by addition of solid sodium carbonate (1.1 g, 10 mmole) and a solution of Fmoc-OSu (2.5 g, 7.4 mmole) in 100 ml of THF. After stirring for 17 hours, the reaction solution was concentrated under reduced pressure to remove THF, and diluted with 50 ml of water. The aqueous solution was washed with DCM (80 ml x 3), acidified to pH 2 by adding concentrated hydrochloric acid, and extracted with EtOAc (150 ml x 3). The combined EtOAc extracts were washed with 1 M hydrochloric acid solution (100 ml x 4) and saturated sodium chloride aqueous solution (100 ml), and dried over magnesium sulfate overnight. The dry EtOAc solution was filtered, and concentrated under reduced pressure to a small volume (ca. 20 ml) and hexane (100 ml) was added to the residue. The white solid which formed was collected by filtration and then recrystallized with MeOH-water to give white powders (**2**, 1.55 g). Yield: 85%. TLC R_f = 0.34 ($CHCl_3$-MeOH-HOAc = 50:5:2). RP-HPLC analysis: retention time = 10.93 min (average of two runs). ^{1}H-NMR (90 MHz, CD_3OD) δ ppm: 8.37 (s, 1H), 7.77-7.17 (m, 8H), and 4.42-4.09 (m, 5H). ESI-MS (m/z): 364.9 [M + H]$^+$, calculated monoisotopic mass 365.11.

Synthesis of 2-(2'-Fmoc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylic acid

(3)

Boc-Ser(Trt)-OH (**29**)

A solution of Fmoc-Ser(Trt)-OH (80 g, 0.14 mole) in 200 ml of DCM and 150 ml of diethylamine was stirred for three hours. The solution was concentrated under reduced pressure to leave a residue. The residue was dissolved in a solution of sodium hydroxide (5.6 g, 0.14 mole) in 50 ml of water and 200 ml of saturated aqueous sodium bicarbonate was added. The solution was washed with petroleum ether (300 ml x 3) and diluted with 100 ml of *t*-butanol. To the resulting stirred solution, di-*t*-butyl dicarbonate (50 g, 0.22 mole) was added dropwise over a period of 30 min. After 15 min, an additional 100 ml of *t*-butanol was added to the reaction mixture, and it was stirred overnight. The solution was then diluted with 200 ml of water, washed with petroleum ether (400 ml x 3), and cooled to 0°C. After three hours, the chilled solution was acidified to pH 3 with 1 N hydrochloric acid and extracted with EtOAc (600 ml x 4). The combined EtOAc extracts was dried (magnesium sulfate) overnight, filtered, and concentrated under reduced pressure to leave a residue. The residue was purified by silica gel column chromatography (40 x 5 cm, solvent system: petroleum ether-EtOAc = 4:1) to afford an oil, which was recrystallized from DCM-hexane to give crystals of (**29**) (49 g) . Yield: 78%. TLC R_f = 0.38 ($CHCl_3$-MeOH-HOAc = 100: 5: 1). ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 10.86 (s, br, 1 H), 7.5-7.1 (m, 15 H), 5.35 (d, 1 H, J = 8.3 Hz), 4.41 (m, 1 H), 3.61 (dd, 1 H, J = 9.1 and 3.3 Hz), 3.38 (dd, 1 H, J = 9.1 and 3.4 Hz), and 1.44 (s, 9 H).

Boc-Ser(Trt)-*N*-methoxy-*N*-methyl amide (**30**)

To a well stirred solution of Boc-Ser(Trt)-OH **29** (22.4 g, 50 mmole) and BOP (22.11 g, 50 mmole) in 100 ml of DCM, was added DIEA (8.7 ml, 50 mmole). The resulting solution was stirred at room temperature for 10 min. A solution of *O*, *N*-dimethylhydroxylamine hydrochloride (5.85 g, 60 mmole) and DIEA (15.66 ml, 90 mmole) in 60 ml of DCM was then added, and stirred for 20 min. The reaction mixture was concentrated under reduced pressure to afford a residue. The residue was purified by silica gel column chromatography (50 x 4 cm, solvent system: hexane-EtOAc = 5:1 and 3:1) to give a colorless oil **30** (24 g). Yield: 98%. TLC R_f = 0.30 (hexane-EtOAc = 3:1). ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 7.5-7.1 (m, 15 H), 5.48 (d, 1 H, J = 8.8 Hz), 4.84 (m, 1 H), 3.57 (s, 3 H), 3.31 (d, 2 H, J = 4.7 Hz), 3.18 (s, 3 H), and 1.43 (s, 9 H).

N^{α}-Boc-O-trityl-L-serinal (**31**)

Boc-Ser(Trt)-*N*-methoxy-*N*-methyl amide (**30**) (24 g, 49 mmole) in 400 ml of

anhydrous diethyl ether was stirred in a ice-water bath under argon for 30 min. A commercially available LAH solution (1 M) in diethyl ether (100 ml, 0.1 mole) was added to the above well stirred solution by cannula under argon. The resulting solution was stirred for 30 min at 0°C. A solution of potassium hydrogensulfate (11.92 g, 87.5 mmole) in 200 ml of water was added to the reaction mixture, and it was stirred for 15 min. The reaction mixture was then diluted with 400 ml of diethyl ether. The organic layer was set aside and the aqueous layer was extracted with diethyl ether (300 ml x 2). The combined ether extracts were washed with 1 M hydrochloric acid (500 ml x 4), saturated sodium bicarbonate (500 ml x 2), saturated sodium chloride (400 ml x 2), dried with 16 g of magnesium sulfate overnight, and filtered. Concentration of the solvent under reduced pressure left an oil **31** (19.94 g) which was used in the next step without further purification. Yield: 94%. TLC R_f = 0.58 (hexane-EtOAc = 2:1). ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 9.52 (s, 1 H), 7.5-7.1 (m, 15 H), 5.27 (s, br, 1 H), 4.32 (m, 1 H), 3.55 (t, 2 H), and 1.45 (s, 9 H).

<u>Methyl 2-(1'-Boc-amino-2'-trityl-*O*-hydroxyethyl)-thiazolidine-4-carboxylate (**32**)</u>

To a stirred solution of N^{α}-Boc-*O*-trityl-L-serinal (**31**) (19.94 g, 46.2 mmole) in 250 ml of DCM, was added a solution of L-Cys-OMe hydrochloride (9.4 g, 54 mmole) and DIEA (15 ml, 86 mmole) in 150 ml of DCM dropwise. TLC monitoring result showed that the reaction was not finished after stirring for 12 hours. Evaporation of the reaction solution under reduced pressure followed by addition of benzene (200 ml). The reaction was finished by the third cycle of addition and removal of benzene under reduced pressure to afford the desired product. The product was purified by silica gel column chromatography (40 x 5 cm, solvent system: hexane-EtOAc = 4:1 and 3:1) to give **32** (24 g). **32** is a diastereomeric mixture which had two spots (ca. 1:1) with R_f 0.42 and 0.45 on TLC (hexane-EtOAc = 2:1). Yield: 95%. ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 7.5-7.1 (m, 15 H), 5.2-4.7 (m, 2 H), 4.4-3.8 (m, 2 H), 3.72 (s, 3 H), 3.4-2.5 (m, 5 H), and 1.45 (s, 9 H).

<u>Methyl 2-(1'-Boc-amino-2'-trityl-*O*-hydroxyethyl)-thiazole-4-carboxylate (**33**)</u>

A solution of methyl 2-(1'-Boc-amino-2'-trityl-*O*-hydroxyethyl)-thiazolidine-4-carboxylate (**32**) (24 g, 43.7 mmole) in 400 ml of benzene was heated to 50°C. To above stirred solution, was added manganese (IV) oxide (activated) (118 g, 30 eq) and pyridine (4 ml). The reaction solution was stirred at 50°-55°C for 5 hours (one of the isomers was

quickly oxidized to the product, while the other was very resistant to the oxidation conditions). After filtration, the insoluble material was washed with DCM (100 ml x 2). The combined filtrates were concentrated under reduced pressure and the residue was purified by silica gel column chromatography (40 x 5 cm, hexane-EtOAc = 4:1) to give **33** (14 g). Yield: 59%. TLC R_f = 0.48 (hexane-EtOAc = 2:1). ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 8.06 (s, 1 H), 7.5-7.1 (m, 15 H), 5.61 (d, 1 H, J = 7.7 Hz), 5.17 (m, 1 H), 3.88 (s, 3 H), 3.78 (dd, 1 H, J = 4.1 and 9.1 Hz), 3.46 (dd, 1 H, J = 9.2 and 4.1 Hz), and 1.42 (s, 9 H).

Methyl 2-(1'-amino-2'-hydroxyethyl)-thiazole-4-carboxylate (**34**)

Methyl 2-(1'-Boc-amino-2'-trityl-*O*-hydroxyethyl)-thiazole-4-carboxylate **33** (14 g, 25 mmole) was added to 200 ml of TFA/triethylsilane/DCM (50:10:40) and stirred for 45 min. The solution was concentrated under reduced pressure to leave a residue, to which 150 ml of 0.1 M hydrochloric acid was added. This acidic solution was washed with DCM (100 ml x 4), neutralized to pH 9 by adding saturated sodium carbonate (aqueous solution), and concentrated to dry at room temperature *in vacuo*. The residue was purified on a Sephadex LH-20 column (50 x 2.5 cm) (eluent: methanol) to afford **34** (4.5 g). Yield: 90%. TLC R_f = 0.16 ($CHCl_3$-MeOH-HOAc = 50: 5: 2). ^{1}H-NMR (90 MHz, CD_3OD) δ ppm: 8.26 (s, 1H), 4.26 (dd, 1H, J = 4.8 and 6.2 Hz), 3.88 (s, 3H), 3.86 (dd, 1H, J = 10.7 and 4.8 Hz), and 3.69 (dd, 1H, J = 10.7 and 6.2 HZ).

Methyl 2-(2'-Boc-aminomethyl-oxazoline-4'-yl)-thiazole-4-carboxylate (**35**)

Methyl 2-(1'-amino-2'-hydroxyethyl)-thiazole-4-carboxylate (**34**) (4.5 g, 22.5 mmole) was dissolved in 30 ml of 1 M hydrochloric acid (aqueous solution) and concentrated at room temperature to dry *in vacuo*. The hydrochloride salt **34a** was then added to a solution of Boc-aminoacetimino ethyl ether (see preparation of **25**, from Boc-glycine amide 10.45 g, 60 mmole) in 100 ml of DCM. The reaction mixture was stirred at room temperature for 24 hours, followed by removal of the solvent under reduced pressure. The residue was purified by silica gel column chromatography (40 x 5 cm, solvent system: hexane-acetone gradient) to yield **35** (3.43 g). Yield: 44%. TLC R_f = 0.39 (hexane-acetone = 1:1). ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 8.10 (s, 1H), 5.53 (dd, 1H, J = 7.9 and 9.6 Hz), 5.17 (s, br, 1H), 4.85-4.40 (m, 2H), 4.03 (d, 2H, J = 5.7 Hz), 3.93 (s, 3H), and 1.46 (s, 9H).

Methyl 2-(2'-Boc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylate (**36**)

To a stirred solution of methyl 2-(2'-Boc-aminomethyl-oxazoline-4'-yl)-thiazole-

4-carboxylate (**35**) (3.43 g, 10 mmole) in 100 ml of benzene at 70°C, nickel peroxide (5 g, 1.6 eq. of active O_2) was added and stirred for 10 hours at 70°C. The solid material was removed by filtration and the filtrate was concentrated under reduced pressure to leave a residue, which was purified by silica gel chromatography (10 x 2.5 cm, solvent system: hexane-acetone = 2:1). The fractions containing **36** were pooled and recrystallized in DCM-MeOH to yield **36** (0.76 g). Yield: 22%. TLC $R_f = 0.70$ (hexane-acetone = 1:1). ^{1}H-NMR (90 MHz, $CDCl_3$) δ ppm: 8.28 (s, 1H), 8.15 (s, 1H), 5.07 (s, br, 1H), 4.48 (d, 2H, J = 6.2 Hz), 3.95 (s, 3H), and 1.47 (s, 9H).

<u>2-(2'-Boc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylic acid (37)</u>

Methyl 2-(2'-Boc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylate (**36**) (0.76 g, 2.2 mmole) was dissolved in 30 ml of THF and 20 ml of 1 M sodium hydroxide (aqueous solution) and stirred at room temperature for 2 hours. The solution was concentrated under reduced pressure to remove the THF. The residue was diluted with 100 ml of water, washed with DCM (50 ml x 3), acidified to pH 2 by adding 10% potassium hydrogensulfate (aqueous solution), and extracted with EtOAc (100 ml x 4). The EtOAc extracts were dried (magnesium sulfate) overnight, filtered, and concentrated under reduced pressure to a small volume to give a powder **37** (0.65 g). Yield: 90%. ^{1}H-NMR (90 MHz, CD_3OD) δ ppm: 8.46 (s, 1 H), 8.34 (s, 1H), 4.40 (s, 2H), and 1.47 (s, 9H).

<u>2-(2'-Fmoc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylic acid (3)</u>

2-(2'-Boc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylic acid (**37**) (0.65 g, 2 mmol) was added in 30 ml of 40%TFA in DCM and stirred for 60 min. The solvent was removed by concentration under reduced pressure. The residue was diluted with 10 ml of water, which was neutralized to pH 7 by adding 1 M sodium hydroxide (aqueous solution), followed by addition of solid sodium carbonate (0.42 g, 4 mmole) and a solution of Fmoc-OSu (1 g, 3 mmole) in 60 ml of THF. After stirring for 2 hours (white solid precipitated), the reaction mixture was concentrated under reduced pressure to remove the THF, and diluted with 30 ml of water. The aqueous solution was washed with DCM (50 ml x 3), and acidified to pH 3 by adding 10% potassium hydrogensulfate (aqueous solution). The white solid which formed was collected by filtration. This product was insoluble in DCM, ether, EtOAc, THF, methanol, ethanol, acetonitrile, HFIP, DMF, NMP and water. It was soluble in DMSO or the solutions containing more than 50% DMSO in DMF or NMP. The product was recrystallized in DMSO-water to

give a fine white powder (0.69 g). Yield: 77%. RP-HPLC analysis: retention tLneime = 14.18 min (average of two runs). ^{1}H-NMR (90 MHz, DMSO-d_6) δ ppm: 8.74 (s, 1H), 8.38 (s, 1H), 7.89-7.31 (m, 8H), and 4.39-4.30 (m, 5H). ESI-MS (m/z): 448.2 $[M + H]^+$, (calculated monoisotopic mass 448.10), 470.2 $[M + Na]^+$, 486.2 $[M + K]^+$.

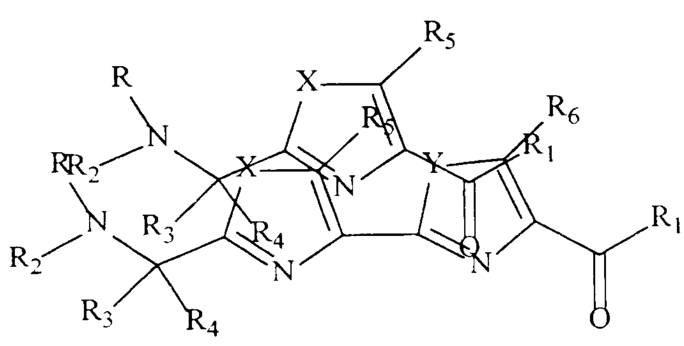
Compound (3) exhibits unexpected properties.

Synthesis of Fmoc-glutamine **(38)**

To a stirred solution of L-glutamine (1.75 g, 12 mmole) and sodium carbonate (1.27 g, 12 mmole) in 30 ml of water, was added a solution of Fmoc-OSu (3.0 g, 8.9 mmole) in 60 ml of THF. After stirring overnight the mixture was concentrated under reduced pressure to remove the THF. The residue was diluted with 100 ml of 1 N hydrochloric acid . The white solid was collected by filtration and recrystallized in DMF-0.1 N hydrochloric acid aqueous solution to afford a white powder (**38**, 2.76 g). Yield: 84%. ^{1}H-NMR (90 MHz, DMSO-d_6) □ ppm: 12.47 (1H, s), 7.89-7.20 (10 H, m), 6.68 (1H, s, br), 4.22-3.90 (4H, m), and 2.25-1.86 (4H, m).

Combinatorial Synthesis

In one embodiment, the present invention relates to the generation of a synthetic combinatorial library of at least two compounds, each compound within the library being derived from the solid phase combinatorial synthesis of at least one compound selected from the group consisting of:



(11)

(12)

where R= H, a naturally occurring or synthetic **L** or **D** amino acid, *Tert-*butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), carbobenzoxy (Z),

Benzozyl (Bz), and other like amino protecting groups;

where R_1 = OH, alkyl esters, aromatic esters such as methyl, ethyl, *t*-butyl and benzyl, activated esters such as pentafluorophenyl, nitrophenyl, N-hydroxysuccinimide, acid chlorides, fluorides, organic salts, such as cyclohexyamines (CHA), amides, an amide bonded to a linker such as a diamine, or an insoluble support for use in solid phase synthesis;

where R_2 = H, a C_1-C_{10} alkyl, or an aromatic ring;

where $R_{3\text{-}4}$ = H, or a C_1-C_{10} alkyl;

where $R_{5\text{-}6}$ = H, C_1-C_{10} alkyl, a heterocylic ring, an aliphatic or aromatic ring, a functional group such as an amine, an alchohol, a halide or an organometallic complex;

where X = oxygen (O) or sulfur (S);

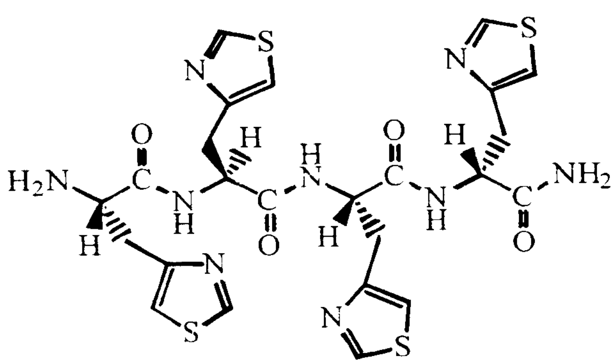
where Y = oxygen (O) or sulfur (S);

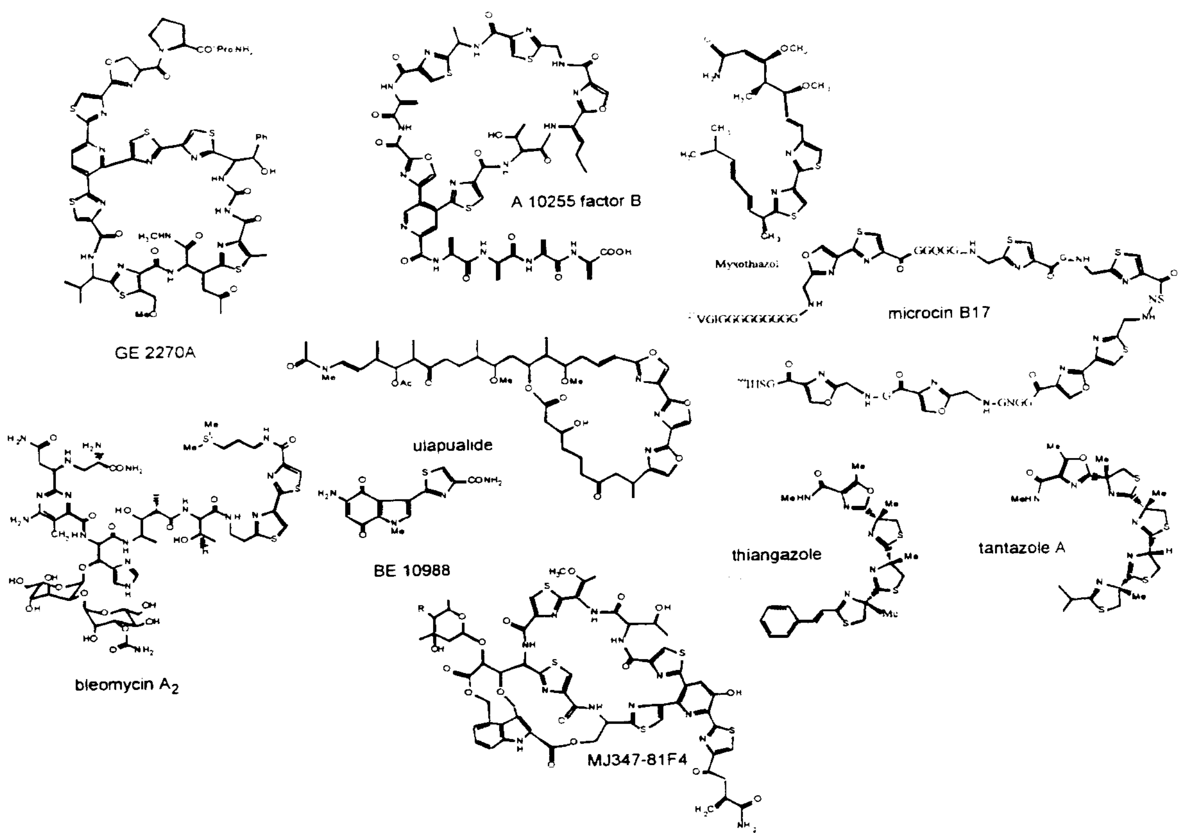
wherein at least one of the compounds selected from the group of **11** and **12** forms an amide bond with at least one of the compounds selected from the group of **11** and **12** or a naturally occurring or synthetic amino acid.

In another embodiment of the invention, at least one of the compounds **11** and **12** is combined with a natural amino acid in a combinatorial synthesis to yield a naturally occurring antibiotic compound.

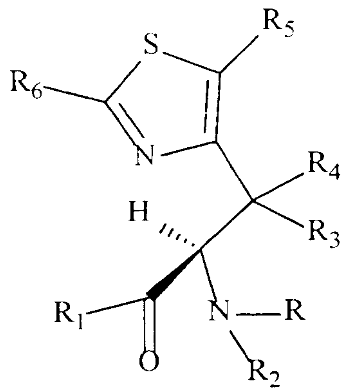
In a preferred embodiment of the invention, at least one the compounds selected from the group **11** and **12** is combined with a natural amino acid in combinatorial synthesis to yield a microcin B17 fragment.

The following compounds are compounds especially believed to be suitable for purposes of the invention.





Another embodiment of the invention relates to the generation of a synthetic combinatorial library of at least two compounds, each compound within the library being derived from the solid phase combinatorial synthesis of at least one compound selected from the group consisting of:



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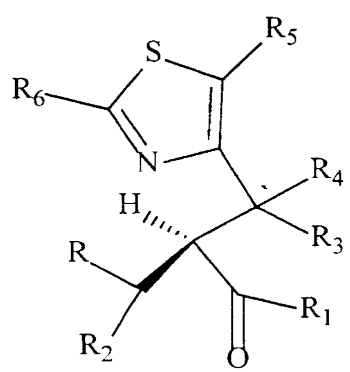


(14)

where R= H, a naturally occurring or synthetic **L** or **D** amino acid, *Tert*-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), carbobenzoxy (Z), Benzozyl (Bz), and other like amino protecting groups;

where R_1 = OH, alkyl esters, aromatic esters such as methyl, ethyl, *t*-butyl and benzyl, activated esters such as pentafluorophenyl, nitrophenyl, N-hydroxysuccinimide, acid chlorides, fluorides, organic salts, such as cyclohexyamines (CHA), amides, an amide bonded to a linker such as a diamine, or an insoluble support for use in solid phase synthesis;

where R_2 = H, a C_1-C_{10} alkyl or an aromatic ring;

where R_{3-4} = H, or a C_1-C_{10} alkyl;

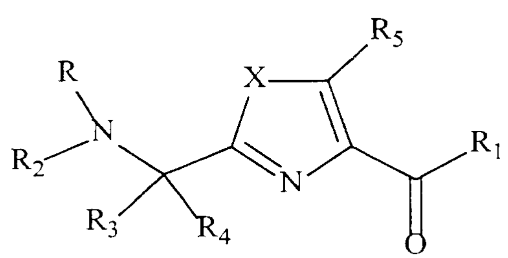
where $R_{5\text{-}6}$ = H, C_1-C_{10} alkyl, a heterocylic ring, an aliphatic or aromatic ring, a functional group such as an amine, an alchohol, a halide or an organometallic complex:

wherein at least one of the compounds selected from the group of **13** and **14** forms an amide bond with at least one of the compounds selected from the group of **13** and **14** or a naturally occurring or synthetic amino acid.

Because libraries can be screened while still bound to the resin, additional embodiments of the invention include any of the above-described libraries bound to a solid-phase resin.

Although certain structures have been shown, enantiomers of those structures are within the scope of the invention.

In yet another embodiment of the invention, a method for the preparation of a library of antibiotic compounds comprises coupling an amino protected first amino acid to a resin, the first amino acid selected from the group consisting of:



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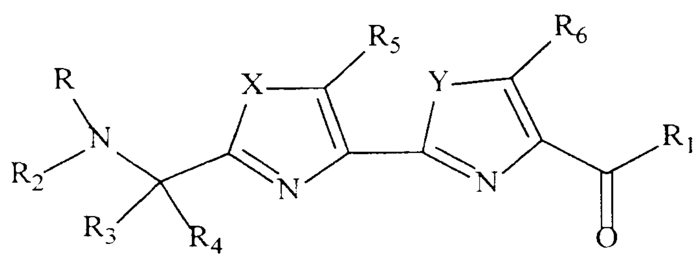


(12)

where R= H, a naturally occurring or synthetic **L** or **D** amino acid, *Tert*-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), carbobenzoxy (Z), Benzozyl (Bz), and other like amino protecting groups:

where R_1 = OH, alkyl esters, aromatic esters such as methyl, ethyl, *t*-butyl and benzyl, activated esters such as pentafluorophenyl, nitrophenyl, N-hydroxysuccinimide, acid chlorides, fluorides, organic salts, such as cyclohexyamines (CHA), amides, an amide bonded to a linker such as a diamine, or an insoluble support for use in solid phase synthesis;

where R_2 = H, a C_1-C_{10} alkyl or an aromatic ring;

where $R_{3\text{-}4}$ = H, or a C_1-C_{10} alkyl;

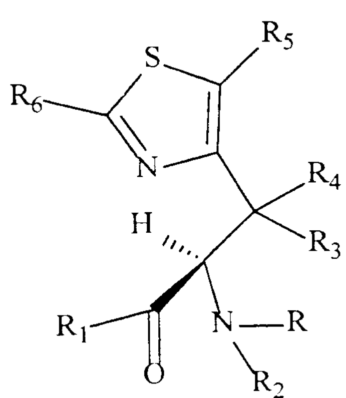
where $R_{5\text{-}6}$ = H, C_1-C_{10} alkyl, a heterocylic ring, an aliphatic or aromatic ring, a functional group such as an amine, an alchohol, a halide or an organometallic complex;

where X = oxygen (O) or sulfur (S);

where Y = oxygen (O) or sulfur (S);

removing the protecting group of the first amino acid, coupling an amino protected second amino acid selected from the group consisting of **11** and **12** or a naturally occurring or synthetic amino acid and cyclizing the compounds selected from the group consisting of **11** and **12** or a naturally occurring or synthetic amino acid from the step of coupling.

In yet another embodiment of the invention, a method for the preparation of a library of antibiotic compounds comprises coupling an amino protected first amino acid to a resin, the first amino acid selected from the group consisting of:



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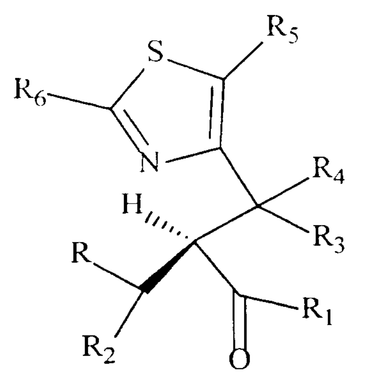


(14)

where R= H, a naturally occurring or synthetic **L** or **D** amino acid, *Tert*-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), carbobenzoxy (Z), Benzozyl (Bz), and other like amino protecting groups;

where R_1 = OH, alkyl esters, aromatic esters such as methyl, ethyl, *t*-butyl and benzyl, activated esters such as pentafluorophenyl, nitrophenyl, N-hydroxysuccinimide, acid chlorides, fluorides, organic salts, such as cyclohexyamines (CHA), amides, an amide bonded to a linker such as a diamine, or an insoluble support for use in solid phase synthesis;

where R_2 = H, a C_1-C_{10} alkyl or an aromatic ring;

where $R_{3\text{-}4}$ = H, or a C_1-C_{10} alkyl;

where $R_{5\text{-}6}$ = H, C_1-C_{10} alkyl, a heterocylic ring, an aliphatic or aromatic ring, a functional group such as an amine, an alchohol, a halide or an organometallic complex; removing the protecting group of the first amino acid, coupling an amino protected second amino acid selected from the group consisting of **3** and **4** or a naturally occurring or synthetic amino acid, and cyclizing the compounds selected from the group consisting of **3** and **4** or a naturally occurring or synthetic amino acid from the step of coupling.

Results and Discussion

Synthesis of library one (**L1**)

Table 1 shows the pair of enantiomeric thiazole-containing unnatural amino acids, **13** and **14**, that were chosen as the building blocks to synthesize a library of sixteen tetrapeptide amides. The thiazole ring is on the side chain of the amino acids.

Table 1: The Sequences of peptide amides of **L1**

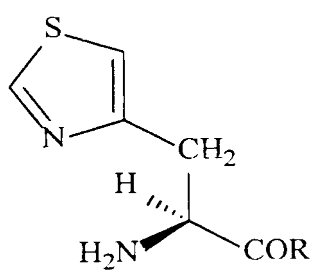
1	DDDD-	5	DDLL-	9	DLLL-	13	DLDD-

	NH_2[a]		NH_2		NH_2		NH_2
2	DDDL-NH_2	6	DDLD-NH_2	10	DLDL-NH_2	14	DLLD-NH_2
3	LDDL-NH_2	7	LDLL-NH_2	11	LLDL-NH_2	15	LLLD-NH_2
4	LDDD-NH_2	8	LDLD-NH_2	12	LLLL-NH_2	16	LLDD-NH_2

D: D-(3)-(4-thiazolyl) alanine (R)

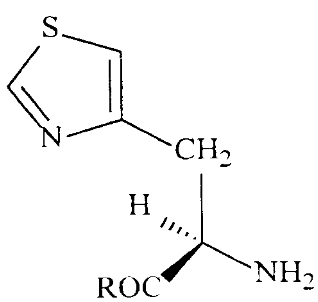
L: L-(3)-(4-thiazolyl) alanine (S)

[a]The linkage between monomers is amide bond.



(13)

L-(3)-(4-thiazolyl)alanine



(14)

D-(3)-(4-thiazolyl) alanine

Peptide amide was synthesized on MBHA resin by Boc strategy. Sixteen syringes of a MULTIBLOCK simultaneous multiple peptide synthesizer were used for this library synthesis.

Boc-amino acids were coupled to the resin by four equivalents of amino acid under the activation of four equivalents of DCC in DCM for 60 min. Ninhydrin test showed that the coupling reaction was satisfactory and there was no need for a second coupling. Boc was removed in 40% TFA in DCM for 30 min.

After the sequences were synthesized, the resin was washed and dried *in vacuo*. The dried peptide resin was cleaved with HF at 0°C for 60 min without adding any scavenger.

HPLC analysis results showed that each individual compound had one peak at 215 nm, indicating that each compound in the library is in high purity.

Because the process and conditions of library synthesis was identical to individual compound in this library and the compounds are either enantiomers or diastereomers, compound **L1-4** (LDDD-NH_2) was chosen as an example to analyze its structure. ^{1}H-NMR spectrum of **L1-4** showed the signals of four thiazolyl aromatic protons at δ 8.88-8.87 (4H, m), 7.29 (1H, d, J = 1.85 Hz), 7.26 (1H, d, J = 1.82 Hz), 7.22 (1H, d, J = 1.83 Hz), and 7.16 (1H, d, J = 1.82 Hz), indicating that the thiazole ring was intact under solid phase synthesis conditions and HF cleavage. The ESI-MS showed the molecular ion peak at 634.1 $[M+1]^+$, (calculated monoisotopic mass 634.11), confirmed the integrity of this peptide. The UV spectrum showed a typical maximum absorbance of thiazole ring at 238 nm (31).

<u>Synthesis of library two (**L2**)</u>

2-Fmoc-aminomethyl-thiazole-4-carboxylic acid (**1**), 2-Fmoc-amino-methyl-oxazole-4-carboxylic acid (**2**), and 2-(2'-Fmoc-aminomethyloxazole-4'-yl)-thiazole-4-carboxylic acid (**3**) were synthesized as previously discussed.

<u>Table 2: The sequences of the compounds in library two (**L2**)</u>

1	Ac-AGA-NH-$(CH_2)_3$-NH_2[a]	9	Ac-AGA-NH-$(CH_2)_3$-NH_2
2	Ac-AGA-NH-$(CH_2)_3$-NH_2	10	Ac-AGA-NH-$(CH_2)_3$-NH_2
3	Ac-AGA-NH-$(CH_2)_3$-NH_2	11	Ac-AGA-NH-$(CH_2)_3$-NH_2
4	Ac-AGA-NH-$(CH_2)_3$-NH_2	12	Ac-AGA-NH-$(CH_2)_3$-NH_2
5	Ac-AGA-NH-$(CH_2)_3$-NH_2	13	Ac-AGA-NH-$(CH_2)_3$-NH_2
6	Ac-AGA-NH-$(CH_2)_3$-NH_2	14	Ac-AGA-NH-$(CH_2)_3$-NH_2
7	Ac-AGA-NH-$(CH_2)_3$-NH_2	15	Ac-AGA-NH-$(CH_2)_3$-NH_2
8	Ac-AGA-NH-$(CH_2)_3$-NH_2	16	Ac-AGA-NH-$(CH_2)_3$-NH_2

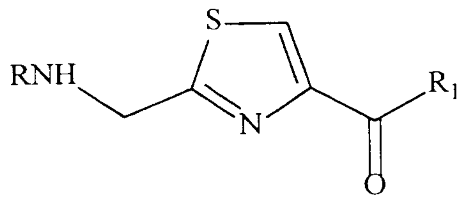
[a]The linkage between monomers is amide bond.

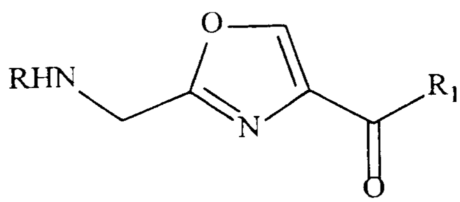
A: 2-Aminomethyl-thiazole-4-carboxylic acid

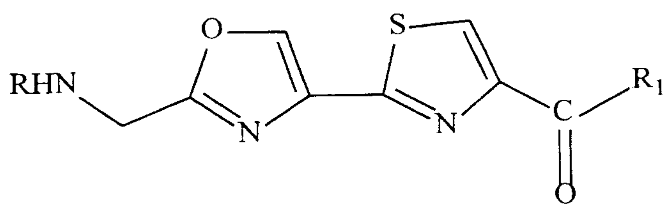
B: 2-Aminomethyl-oxazole-4-carboxylic acid

C: 2-(2`-Aminomethyl-oxazole-4`-yl)-thiazole-4-carboxylic acid

G: glycine

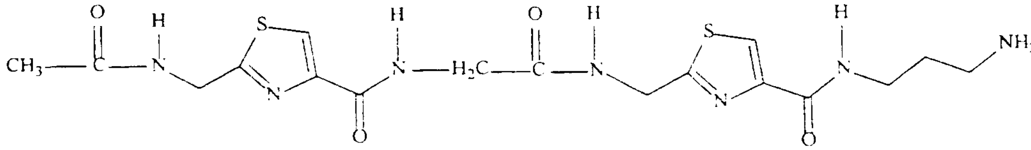
A: 

B: 

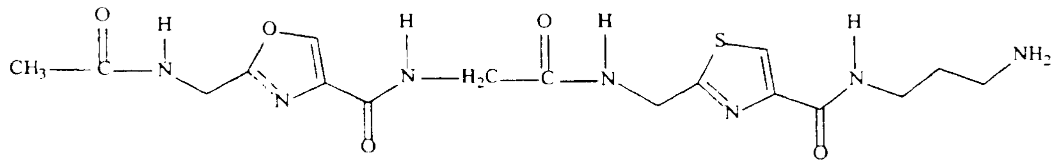
C: 

where R= H and R_1 = OH

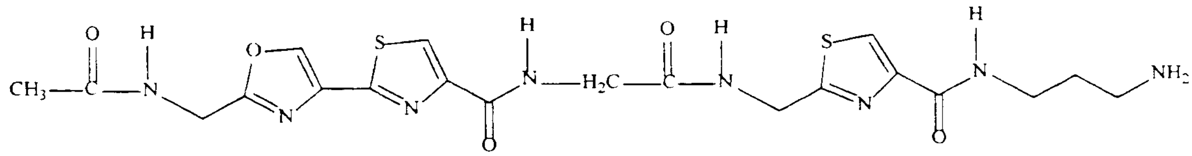
The following compounds from the library **L2** were identified by using UV spectrum, ESI-MS and RP-HPCL technology:



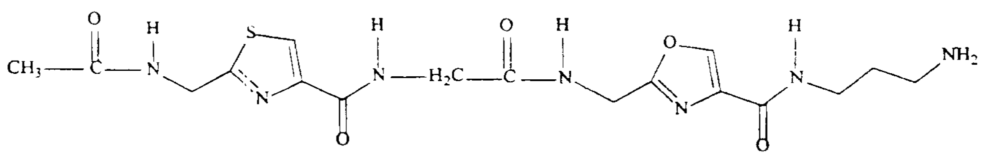
(L2-1)



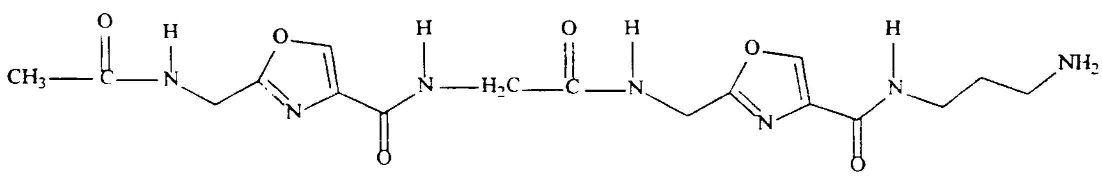
(L2-2)



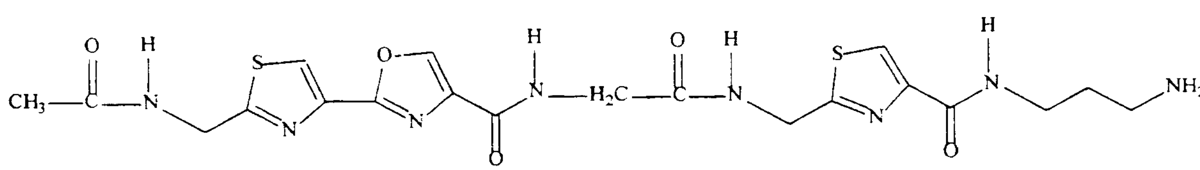
(L2-3)



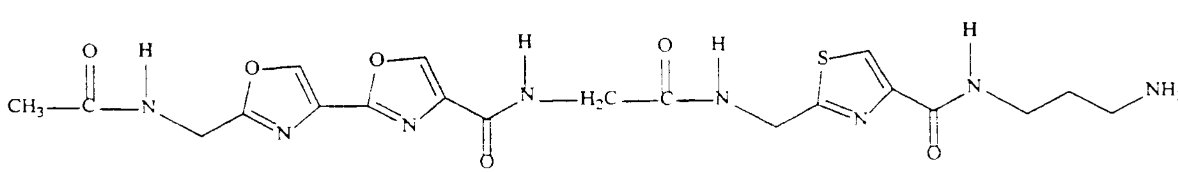
(L2-4)



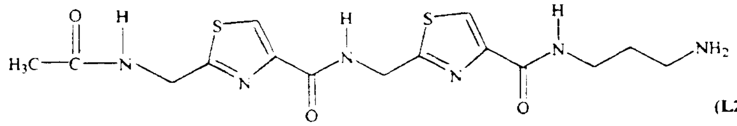
(L2-5)



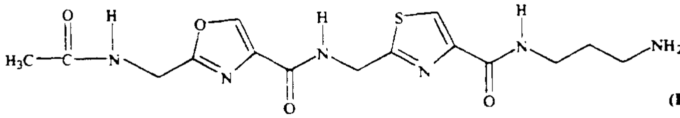
(L2-7)



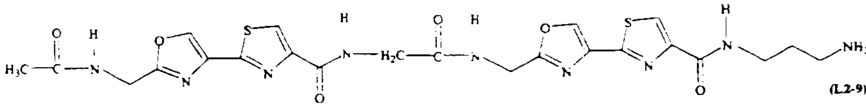
(L2-8)



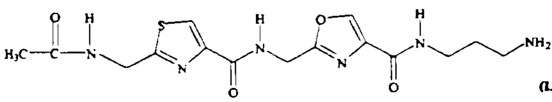
(L2-10)



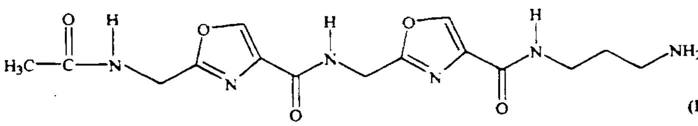
(L2-11)



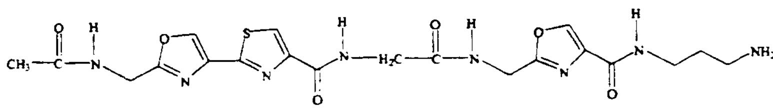
(L2-9)



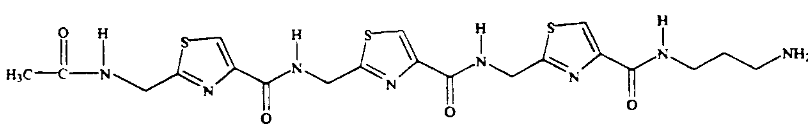
(L2-12)



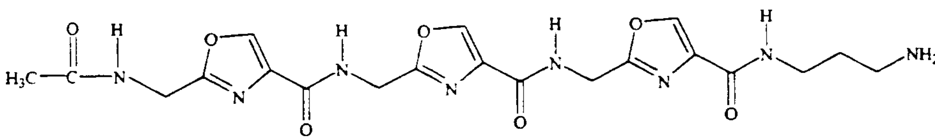
(L2-13)



(L2-6)



(L2-14)



(L2-15)

Compounds **L2-1** to **L2-9** were the combinations of three building blocks with a glycine insertion between two building blocks in each compound. The glycine addition was designed to increase the flexibility of the peptide. **L2-10** to **L2-13** were the combinations of building blocks **1** and **2**, in which **3** was not used. Otherwise, it was thought that the structures of peptides would be too rigid. Compounds **L2-14** and **L2-15** were designed to compare with **L2-10** and **L2-13**.

This library was synthesized on 1,3-diaminopropane trityl resin by Fmoc strategy. Therefore, the C-terminal of each compound has a propylamine unit. Fifteen syringes of a MULTIBLOCK simultaneous multiple peptide synthesizer were used for this library synthesis.

Coupling reaction was performed by 1.5 equivalents of Fmoc-amino acid: BOP: HOBt: DIEA (1: 1 :1 :1) with 10-min preactivation before coupling. Although it was shown that the coupling completion was very rapid (less than 10 min) (16), a long reaction time (60 min) was applied to coupling for only 1.5 equivalents of amino acids was added in the reaction. Ninhydrin test indicated that the coupling was satisfactory. Fmoc was removed in 20% piperidine in NMP for 20 min.

Building block **3** can not dissolve in NMP. In the coupling step with **3**, NMP-DMSO (3:4) was used to dissolve **3** in coupling solution.

After the sequences were synthesized, the N-terminal of each compound on the resin was acetylated with 3 equivalents of glacial acetic acid: BOP: HOBt : DIEA (1: 1: 1: 1) with 10-min preactivation before coupling.

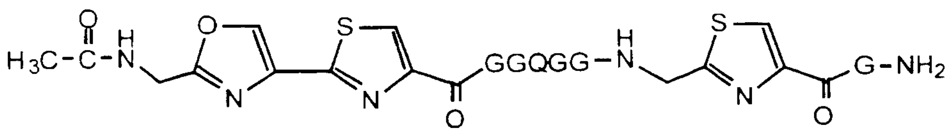
The acetylated resin was washed, dried *in vacuo*, and cleaved with 30%HFIP in DCM at room temperature (30 min). The DCM solution containing cleaved peptide was then dried by blowing nitrogen to leave a residue, which was dissolved in glacial acetic acid and lyophilized.

HPLC analysis showed that individual compound had one peak, indicating these compounds are pure. ESI-MS measured molecular weight for each compound is consistent with the calculated value, confirmed the structure.

This synthesis demonstrated that using only 1.5 equivalents of amino acid, the coupling reaction was quite efficient. Both the yield and purity of product were high (>

80%).

Synthesis of microcin B17 fragment 13-23 (**39**)



The sequence of **39**

Microcin B17 fragment 13-23 (**39**) was synthesized on MBHA resin by Fmoc strategy. 2-Fmoc-aminomethyl-thiazole-4-carboxylic acid (**1**), 2-(2'-Fmoc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylic acid (**3**), and Fmoc-glutamine (**38**) were synthesized in chapter 1. Coupling reaction was performed by two equivalents of Fmoc-amino acid: BOP: HOBt: DIEA (1: 1 :1 :1) with 10-min preactivation before coupling (16).

MBHA resin was used to synthesize peptide amide. Fmoc-amino acid was coupled to the resin for 60 min. Coupling was monitored via a ninhydrin test.

Compound **3** can not dissolve in NMP. In the coupling step with **3**, 1 ml of DMSO was added in coupling solution.

After the sequence was synthesized, and Fmoc group was removed, the resin-bound peptide was acetylated by acetic anhydride (10 eq.) in the presence of DIEA in NMP for two hours and monitored by ninhydrin test.

Then, the resin was washed, dried *in vacuo* overnight, and cleaved with HF at 0°C for 60 min without adding any scavenger.

HPLC analysis of the product showed that there was a main peak at 14.70 min (content >90%) with two minor peaks at 15.23 min (ca. 2%) and 15.66 min (ca. 5%) in the product.

UV spectrum of **39** showed two shoulder peaks at 223sh nm (ε 2.0 x 10^4 M^{-1} cm^{-1}) and 276sh nm (ε 8450). ESI-MS measured molecular weight of **39** is consistent with the calculated monoisotopic mass, confirmed the integrity of this peptide.

Experimental Section

Boc-D-3-(4-thiazolyl) alanine and Boc-L-3-(4-thiazolyl) alanine were purchased from SyntheTech. 1,3-Diaminopropane trityl resin (0.83 mmol/g) and Fmoc-glycine were from Novabiochem. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was from Aldrich. *N*, *N'*-Dicyclohexylcarbodiimide (DCC), *N*, *N*-diisopropylethylamine (DIEA), ninhydrin,

1-hydroxybenzotriazole (HOBt) and acetic anhydride were from Fluka. 4-Methylbenzhydrylamine resin (MBHA, 1.11 mmol/g, 200-400 mesh), trifluoroacetic acid (TFA), benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP), and piperidine were from Advanced ChemTech. *N*-methylpyrrolidone (NMP), acetonitrile (HPLC grade, UV cutoff 189 nm), dimethyl sulfoxide (DMSO), and dichloromethane (DCM) were from Burdick & Jackson. 2-propanol (IPA) was from Fisher. Acetic acid glacial was from Mallinckrodt.

2-(Fmoc-aminomethyl)-thiazole-4-carboxylic acid (**1**), 2-(Fmoc-amino-methyl)-oxazole-4-carboxylic acid (**2**), 2-(2'-Fmoc-aminomethyloxazole-4'-yl)-thiazole-4-carboxylic acid (**3**), and Fmoc-glutamine (**38**) were synthesized in as previously discussed.

<u>Ninhydrin test</u>

Reagent a: Mix solution 1 and solution 2 (solution 1: dissolve 40 g of phenol in 10 ml of absolute ethanol. Stir the solution with 4 g of Amberlite mixed-bed resin MB-3 for 45 min. Filter; solution 2: dissolve 33 mg of KCN in 50 ml of water. Dilute 2 ml of the KCN solution to 100 ml with pyridine. Stir with 4 g of Amberlite mixed-bed resin MB-3. Filter).

Reagent b: Dissolve 2.5 g of ninhydrin in 50 ml of absolute ethanol. Store in dark under nitrogen.

Procedures: A few beads of resin sample were removed into a test tube from reaction vessel using a glass pipette. The resin was washed with isopropanol by decantation. Four drops of reagent a and two drops of reagent b were added into the test tube and mixed well. The test tube was then placed in a preheated heating block at 100°C for five min. Negative reaction was indicated by white beads and yellow solution through observing the test tube against a white background.

HPLC analyses were performed on a Waters HPLC system using a Vydac 218TP C18 10 μm reversed-phase column (250 x 4.6 mm). 18.2 MΩ water was produced by a Milli-Q plus system (Millipore, Bedford, MA).

UV spectra were recorded on a Hitachi U-2000 spectrophotometer.

The molecular weight of products was determined by ESI-MS at Pfizer Central Research (Groton, CT) on a PE SCIEX API-100B LC/MS System (Foster City, CA). Mode: ESI, single quad. m/z = 300-2200, 4.2 sec/scan, flow rate: 200 μl/min, acetonitrile-water (50:50) in 0.1% TFA (v/v). The data were processed using

BioMultiview 1.3 alpha program.

The ^{1}H NMR spectrum was measured on a Bruker 300 spectrometer, solvent: D_2O. Abbreviations for peak description are s = singlet, d = doublet, and m = multiplet.

Synthesis of library one (**L1**)

This library was synthesized on MBHA resin by Boc strategy. Sixteen syringes of a MULTIBLOCK simultaneous multiple peptide synthesizer were used for this library synthesis.

MBHA resin (40 mg, 0.0444 mmol) was placed in each syringe (10 x 45 mm). The resin in each syringe was washed with DCM (6 x 1.5 ml, 9 min), 10% DIEA in DCM (2 x 1.5 ml, 3 min), and DCM (6 x 1.5 ml, 9 min). According to the sequences in Table 3, Boc-D-3-(4-thiazolyl) alanine or Boc-L-3-(4-thiazolyl) alanine (48.3 mg, 0.1776 mmol, 4 eq.) was dissolved in 1 ml of DCM and added to the syringe (1.5 min). The syringes were shaken for 60 min after 178μl of 1M DCC in DCM (36.7 mg, 0.1776 mmol) was added to each syringe. A resin sample was taken for ninhydrin test. The syringes were then washed with DCM (6 x 1.5 ml, 9 min). The Boc group was removed by shaking the syringe with 40% TFA in DCM (1 x 1 ml, 1.5 min; 1 x 1.5 ml, 30 min), and the deprotection was monitored by ninhydrin test.

Above cycle was repeated to continue the syntheses. After the sequences were synthesized, and Boc group was removed, the resin was washed with DCM (6 x 1.5 ml, 9 min) and dried in vacuum overnight. The dried resin was cleaved with HF at 0°C for 60 min without adding any scavenger. After cleavage, the resin was extracted with 10% acetic acid aqueous solution (4 x 2 ml). The extraction solution was lyophilized to yield the peptide product. The results are depicted in Table 3.

Table 3: The data of the compounds in library one (**L1**).

Compd No.	Structure[a]	Product (mg)	Yield (%)	Retention time[b] (min)
1	DDDD-NH_2	9.9	35.2	8.73
2	DDDL-NH_2	10.0	35.6	9.64
3	LDDL-NH_2	7.7	27.4	10.28
4	LDDD-NH_2	11.0	39.1	9.94
5	DDLL-NH_2	8.7	31.0	9.06

6	DDLD-NH_2	10.6	37.7	10.10
7	LDLL-NH_2	15.1	53.7	10.92
8	LDLD-NH_2	15.6	55.5	11.38
9	DLLL-NH_2	14.3	50.9	9.94
10	DLDL-NH_2	11.8	42.0	11.38
11	LLDL-NH_2	14.2	50.5	10.10
12	LLLL-NH_2	19.4	69.0	8.73
13	DLDD-NH_2	18.3	65.1	10.92
14	DLLD-NH_2	16.9	60.1	10.28
15	LLLD-NH_2	17.5	62.3	9.64
16	LLDD-NH_2	12.9	45.9	9.06

a. D: D-(3)-(4-thiazolyl) alanine, L: L-(3)-(4-thiazolyl) alanine.

b. HPLC system was described in General part. Mobile-phase gradient: 10%-30% acetonitrile in 0.1% (v/v) TFA over 20 min.; Flow rate 1.0 ml/min; UV detection: 215 nm; Sample injection: 5 μl. The retention time was reported in the average of two runs when the enantiomers were co-injected.

The spectroscopic data of **L1-4** (LDDD-NH_2)

^{1}H-NMR (300 MHz, D_2O) δ ppm: 8.88-8.87 (4H, m), 7.29 (1H, d, J = 1.85 Hz), 7.26 (1H, d, J = 1.82 Hz), 7.22 (1H, d, J = 1.83 Hz), 7.16 (1H, d, J = 1.82 Hz), 4.67-4.57 (4H, m), and 3.30-2.92 (8H, m). ESI-MS (m/z): 634.1 $[M+1]^+$, calculated monoisotopic mass 634.11. UV : λ_{max} (H_2O) 238 nm (ε 6500 $M^{-1}cm^{-1}$).

Synthesis of library two (**L2**)

This library was synthesized on 1,3-diaminopropane trityl resin by Fmoc strategy. Fifteen syringes of a MULTIBLOCK simultaneous multiple peptide synthesizer were used for this library synthesis. The syntheses of 2-Fmoc-aminomethyl-thiazole-4-carboxylic acid (**1**), 2-Fmoc-aminomethyl-oxazole-4-carboxylic acid (**2**), and 2-(2'-Fmoc-aminomethyloxazole-4'-yl)-thiazole-4-carboxylic acid (**3**) have been disclosed herein.

Coupling reaction was performed by 1.5 equivalents of Fmoc-amino acid: BOP: HOBt: DIEA (1: 1 :1 :1) with 10-min preactivation before coupling (16).

1,3-Diaminopropane trityl resin (0.83 mmol/g) (150 mg, 0.124 mmol) was placed in each syringe (10 x 45 mm). The resin in each syringe was washed with DCM (3 x 1.5

ml, 6 min), NMP (3 x 1.5 ml, 6 min), 5% DIEA in NMP (2 x 1.5 ml, 3 min), and NMP (6 x 1.5 ml, 9 min).

According to the sequences in Table 2, Fmoc-amino acid (0.186 mmol, 1.5 eq.), BOP (82.3 mg, 0.186 mmol) and HOBt (25.7 mg, 0.186 mmol) were dissolved in 1 ml of NMP, followed by addition of DIEA (32.5 μl, 0.186 mmol). The solution was shaken for 10 min and added to the syringe. The syringes were shaken for 60 min. A resin sample was taken for ninhydrin test. The syringes were then washed with NMP (3 x 1.5 ml, 6 min), DCM-IPA (1:1) (3 x 1.5 ml, 6 min), IPA (3 x 1.5 ml, 6 min), and NMP (3 x 1.5 ml, 9 min). The Fmoc group was removed by shaking the syringe with 20% piperidine in NMP (1 x 1.5 ml, 1.5 min; 1 x 1.5 ml, 20 min), and the deprotection was monitored by ninhydrin test. The syringes were then washed with NMP (3 x 1.5 ml, 6 min), DCM-IPA (1:1) (3 x 1.5 ml, 6 min), IPA (3 x 1.5 ml, 6 min), and NMP (3 x 1.5 ml, 9 min).

Above cycle was repeated to continue the syntheses. Compound **3** was dissolved in 1 ml of NMP-DMSO (3:4). In last cycle of acetylation, glacial acetic acid (21.3 μl, 0.372 mmol, 3 eq.), BOP (164.6 mg, 0.372 mmole), HOBt (51.4 mg, 0.372 mmole and DIEA (97.0 μl, 0.558 mmole, 4.5 eq.) were dissolved in 1 ml of NMP.

After the sequences were synthesized, the resin was washed with NMP (3 x 1.5 ml, 6 min), DCM-IPA (1:1) (3 x 1.5 ml, 6 min), IPA (3 x 1.5 ml, 6 min), and dried in vacuum overnight. The dried resin was cleaved with 30%HFIP in DCM at room temperature (2 ml, 30 min x 3). After the peptide solution was dried by blowing N_2, the residue was dissolved in 4 ml of glacial acetic acid. The acetic acid solution was lyophilized to yield the product. The results are depicted in Table 4.

Table 4: The data of the compounds in library two (**L2**).

Compd No.[a]	Product (mg)	Yield (%)	UV[b] λ_{max} (nm) (ε x10^4)	Retention time (min)[c]	MW $[M+1]^+$ Found[d]	Calculated
1	53.1	83.5	236 (1.67)	5.45	454.2	454.13
2	51.6	83.5	214sh (1.90)	4.21	438.2	438.16
3	57.0	79.2	276sh (1.10)	12.46	521.5	521.14
4	50.5	82.0	214sh (1.93)	4.58	438.2	438.16
5	47.1	79.0	210 (2.30)	3.74	422.2	422.18
6	54.7	78.0	275 (0.82)	10.44	505.2	505.16
7	54.9	72.2	276 (1.06)	12.68	521.5	521.14

8	49.0	70.0	276 (0.60)	9.08	505.2	505.16
9	62.2	77.3	276 (1.46)	17.14	588.4	588.14
10	46.2	81.8	240 (1.67)	7.35	397.3	397.11
11	46.2	84.6	210sh (1.87)	5.02	381.3	381.13
12	48.8	89.4	214sh (1.73)	5.25	381.3	381.13
13	45.5	86.5	214 (2.14)	4.08	364.9	365.16
14	62.8	85.0	240 (3.07)	15.91	537.1	537.12
15	60.5	89.0	213 (3.30)	6.00	489.2	489.18

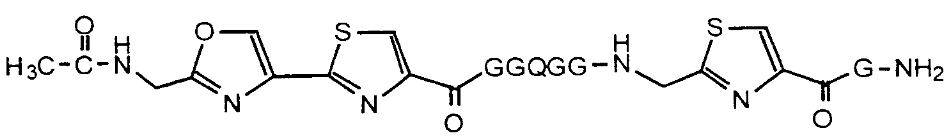
a. The structures see Table 2.

b. Solvent : water; uint of ε: $M^{-1} cm^{-1}$; sh : shoulder peak.

c. HPLC system was described in General part. Mobile-phase gradient: 10%-30% acetonitrile in 0.1% (v/v) TFA over 20 min.; Flow rate 1.0 ml/min; UV detection: 214 nm and the λ_{max}; Sample injection: 5 µl or 2 µl (1 mM). The retention time was reported in the average of two runs.

d. The found molecular weight was determined by ESI-MS. The calculated one is the monoisotopic molecular weight.

<u>Synthesis of microcin B17 fragment 13-23 (**39**)</u>



The sequence of **39**

Microcin B17 fragment 13-23 (**39**) was synthesized on MBHA resin by Fmoc strategy. 2-Fmoc-aminomethyl-thiazole-4-carboxylic acid (**1**), 2-(2'-Fmoc-aminomethyl-oxazole-4'-yl)-thiazole-4-carboxylic acid (**3**), and Fmoc-glutamine (**38**) were synthesized as previously discussed. Coupling reaction was performed by two equivalents of Fmoc-amino acid: BOP: HOBt: DIEA (1: 1 :1 :1) with 10-min preactivation before coupling.

MBHA resin (0.10 g, 0.111 mmol) was placed in a syringe reaction vessel (10 x 45 mm). The resin was washed with NMP (3 x 1.5 ml, 6 min), DCM-IPA (1:1) (3 x 1.5 ml, 6 min), IPA (3 x 1.5 ml , 9 min), and NMP (3 x 1.5 ml, 9 min).

According to the sequence of **39**, Fmoc-amino acid (0.222 mmole, 2 eq.) was dissolved in 444 µl of 0.5 M BOP solution in NMP and 444 µl of 0.5 M HOBt solution

in NMP by vortexing. After 444 μl of 0.5 M DIEA solution in NMP was added to above solution and shaken for 10 min, the solution was added to the reaction vessel.

The reaction vessel was shaken for 60 min. A resin sample was taken for ninhydrin test. The reaction vessel was then washed with NMP (3 x 1.5 ml, 6 min), DCM-IPA (1:1) (3 x 1.5 ml, 6 min), IPA (3 x 1.5 ml, 9 min) and NMP (6 x 1.5 ml, 9 min). The Fmoc group was removed by shaking the vessel with 20% piperidine in NMP (1 x 1.5 ml, 1.5 min; 1 x 1.5 ml, 20 min), and the deprotection was monitored by ninhydrin test.

Above cycle was repeated to continue the synthesis. Compound **3** was dissolved in 1 ml of DMSO, followed by addition of 444 μl of 0.5 M BOP solution in NMP, 444 μl of 0.5 M HOBt solution in NMP, and 39 μl of DIEA.

After the sequence was synthesized, and Fmoc group was removed, the resin-bound peptide was acetylated by shaking reaction vessel with a solution of acetic anhydride (105 μl, 1.11 mmol, 10 eq.) and DIEA (193 μl, 1.11 mmole) in 1.5 ml of NMP for two hours and monitored by ninhydrin test. The resin was washed with NMP (3 x 1.5 ml, 6 min), DCM-IPA (1:1) (3 x 1.5 ml, 6 min), IPA (6 x 1.5 ml, 9 min) and dried in vacuum overnight. The dried resin was cleaved with HF at 0°C for 60 min without adding any scavenger. After cleavage, the resin was extracted with glacial acetic acid (4 x 2 ml). The extraction solution was lyophilized to yield the peptide product (23.8 mg, yield 26%).

HPLC analysis Mobile-phase gradient: 10%-45% acetonitrile in 0.1% (v/v) over 35 min; Flow rate: 1 ml/min; UV detection: 215 and 276 nm; Sample: 5 μl (1 mM) was injected; The retention time of main peak: 14.70 min (purity >90%).

UVλ (H_2O) (nm): 223sh (ε 2.0×10^4 M^{-1} cm^{-1}) and 276sh (ε 8450).

ESI-MS (m/z): 820.3 $[M+1]^+$, calculated monoisotopic molecular weight 820.23.

<u>Bioassay of the peptidomimetic libraries</u>

The thiazole and oxazole-containing peptides from natural sources have important biological activities such as antitumor, antifungal, antibiotic and antiviral activities. To establish if the thiazole and oxazole ring systems could be important pharmacophores in those biologically active peptides, two libraries of thiazole and/or oxazole-containing peptidomimetics, and a microcin B17 fragment **39** were synthesized and found to have antibiotic activity including antibacterial and antifungal.

The DNA binding activity of the tetrapeptide amides in the first library was measured using capillary zone electrophoresis. The results are depicted in Table 5.

Table 5: Binding Constants of the 15 peptide amides in Library 1

Compound	Sequence	$K_a(1)^b$	Compd.	Sequence	$K_a(1)^b$
12	LLLL-NH_2	2.1×10^6	3	LDDL-NH_2	2.0×10^4
9	DLLL-NH_2	4.2×10^5	14	DLLD-NH_2	1.8×10^4
11	LLDL-NH_2	1.9×10^5	10	DLDL-NH_2	1.5×10^4
8	LDLD-NH_2	5.5×10^4	13	DLDD-NH_2	1.4×10^4
15	LLLD-NH_2	5.2×10^4	16	LLDD-NH_2	1.4×10^4
7	LDLL-NH_2	2.8×10^4	1	DDDD-NH_2	2.5×10^4
2	DDDL-NH_2	2.4×10^4	5	DDLL-NH_2	1.7×10^4
6	DDLD-NH_2	2.3×10^4			

Peptides are listed in the order of $K_a(1)$ value from highest to lower value.

$^bK_a(1)$ (M^{-1} is the stoichiometric equilibrium binding constant near saturation.

In addition, peptides **L1-3, L1-5, L1-7, L1-13, L1-14,** and **L1-16** were evaluated for inhibition of the growth of rat hepatoma cell lines 1682A, 1682B, 1683.1.4 and T252. No growth inhibition was observed in both 10% serum and serum-free media.

The method used to determine the cell growth inhibition activity of the compounds in the second library and also a fragment of microcin B17, compound **39** is herein described.

Results and discussion

The marin bacterium *Vibrio anguillarum*, a fish pathogen causing the disease "vibriosis" in marine fish and shellfish were used in this experiment.

Bacteria (*V. anguillarum*) were grown overnight at 28°C in Luria-Bertani (LB) 20 medium (the concentration of NaCl is 20 g/L for marine bacteria in LB medium, instead of the usual 10 g/L). The culture was re-inoculated and incubated in LB20 medium at 28°C (ca. 2hr) to reach the exponential growth phase of the bacteria. The bacterial suspension was then dilute with 0.1 x LB20 medium to make the bacterial dilution containing 2 x 10^3 colony-forming units (cfu)/ml.

At time zero, the bacteria were treated with the peptide in 0.1 x LB20 medium. After incubation for 3 hours, 1 x LB20 medium was added to the culture, and incubated

for 20 hours. The results showed that peptides **L2-6, L2-9** and **39** inhibited the growth of bacterial cultures. However, these peptides did not kill the bacteria, because the increase in the optical density (OD) of the cultures at different incubation times showed the cells were still growing in the cultures.

The antibiotic peptide tachyplesin was used to assess the bacterial assay used. Compared to tachyplesin (Fig. 6 and Table 8), the bacteria treated with peptides **L2, L2-9** and **39** gradually recovered their ability to divide.

The effect of peptides **L2-6, L2-9** and **39** on the growth of *V. anguillarum* is very similar to the effect of microcin B17 on the growth of cells which are immune to microcin B17.

A structural comparison of peptides **L2-6, L2-9** and microcin B17 fragment **39** revealed that they have the identical *N*-terminal moiety, the acetyl-oxazolyl thiazole amino acid building block. Further comparison of these structures with other peptides in the library indicated that the *N*-terminal moiety must not be the only requirement for the activity, because peptide **L2-3** has the same *N*-terminal moiety and **L2-3** did not have a detectable effect on the growth of *V. anguillarum*.

Table 6: OD_{650}[a] of *V. anguillarum* cultures after incubation with peptides **L2-6** and **L2-9** for 9 and 20 hr at 28°

Compund	**L2-6**		**L2-9**		Growth Control	
Incubation time (hr)	9	20	9	20	9	20
No peptide added					0.138	0.400
Dilution 1 (500μM)	0.073	0.388	0.075	0.354		
Dilution 2 (250μM)	0.113	0.427	0.125	0.409		
Dilution 3 (125μM)	0.120	0.402	0.131	0.425		
Dilution 4 (62.5μM)	0.130	0.391	0.135	0.421		
Dilution 5 (31.2μM)	0.137	0.395	0.135	0.417		

[a]OD_{650} is the average of measurements from three different wells.

Table 7: OD_{650}[a] of *V. anguillarum* cultures after incubation with microcin B17 fragment

39 for 9 and 20 hr at 28°

Compound	39		Growth control	
Incubation time (hr)	9	20	9	20
No peptide added			0.126	0.407
Dilution 1 (500μM)	0.034	0.244		
Dilution 2 (250μM)	0.034	0.241		
Dilution 3 (125μM)	0.051	0.279		
Dilution 4 (62.5μM)	0.082	0.297		
Dilution 5 (31.2μM)	0.111	0.419		

[a]OD_{650} is the average of measurements from three different wells.

Table 8: OD_{650}[a] of *V.anguillarum* cultures containing the antibacterial peptide tachyplesin after incubation for 9 and 20 hr at 28°

Compound	tachyplesin		Growth control	
Incubation time (hr)	9	20	9	20
No peptide added			0.170	0.374
Dilution 1 (50 μg/ml)	0.039	0.038		
Dilution 2 (25 μg/ml)	0.035	0.035		
Dilution 3 (12.5μg/ml)	0.035	0.035		
Dilution 4 (6.25 μg/ml)	0.068	0.036		
Dilution 5 (31.2 μg/ml)	0.098	0.034		

[a]OD_{650} is the average of measurements from three different wells.

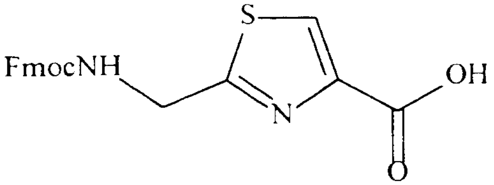
All journal articles and reference citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference.

The foregoing description has been limited to a specific embodiment of the

invention. It will be apparent, however, that variations and modifications can be made to the invention, with the attainment of some or all of the advantages of the invention. Therefore, it is the object of the appended claims to cover all such variations and modifications as come within the true spirit and scope of the invention.

Having described our invention, what we now claim is:

1. A method for the production of a *N*-protected thiazole amino acid comprising the structure of



(1)

which comprises:

effecting a reaction with

BocNH-CH_2-COOH

to produce

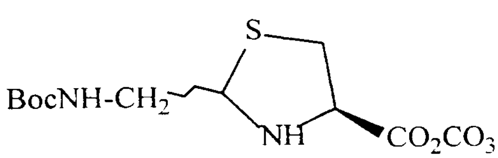
$$\begin{array}{c} \quad\quad\quad\quad\quad\quad OCH_3 \\ \quad\quad\quad\quad\quad\quad | \\ \text{BocNH-CH}_2\text{-CO-N-CH}_3 \end{array}$$

(19)

reducing **(19)** produce

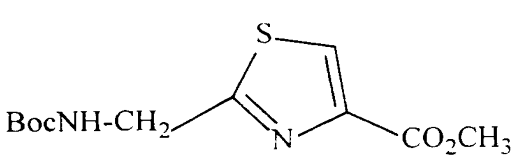
BocNH-CH_2-CHO **(20)**

condensing **(20)** to produce



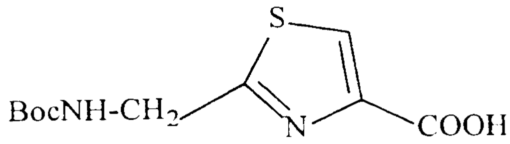
(21)

dehydrogenating **(21)** to produce



(22)

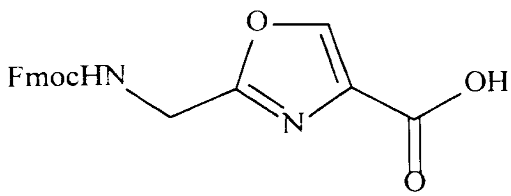
hydrolyzing **(22)** to produce



(23)

removing the boc protecting group of **(23)** to produce **(1)**.

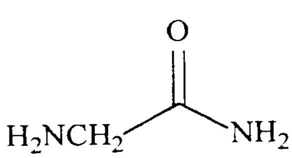
2. An method for the production of a *N*-protected oxazole amino acid comprising the structure of:



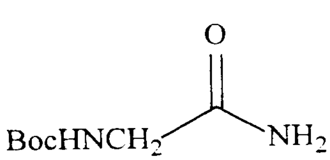
(2)

which comprises:

effecting a reaction with

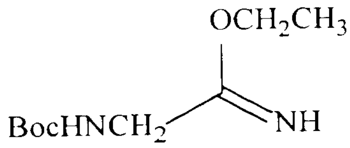


to produce



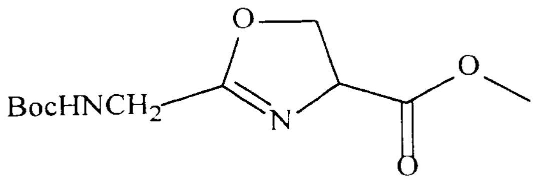
(24)

dissolving **(24)** to produce



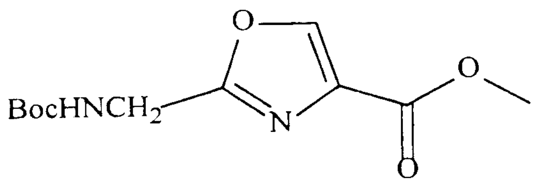
(25)

reacting **(25)** to produce



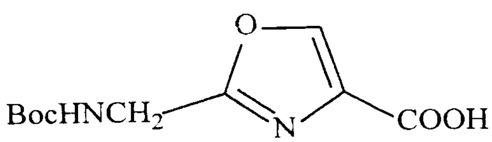
(26)

dehydrogenating **(26)** to produce



(27)

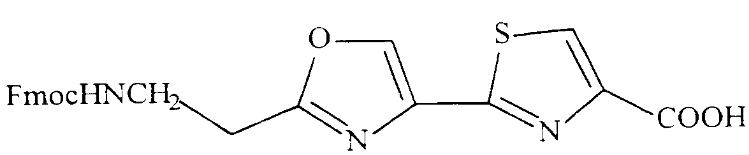
hydrolyzing **(28)** to produce



(27)

removing the Boc protective group of **(27)** to produce **(2)**.

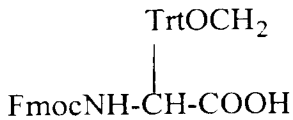
3. An method for producing a *N*-protected oxazole and thiazole amino acid comprising the structure of:



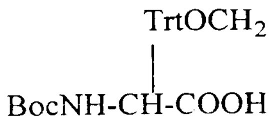
(3)

which comprises:

removing the Fmoc protective group of

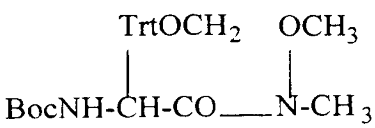


to produce



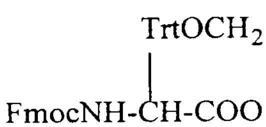
(29)

effecting a reaction with **(29)** to produce



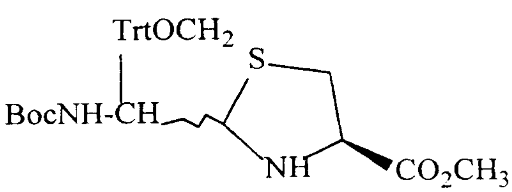
(30)

reducing **(30)** to produce



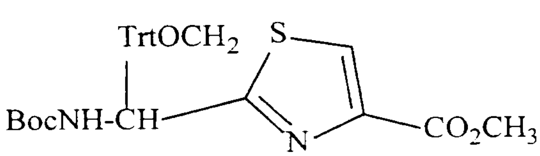
(31)

condensing **(31)** to produce



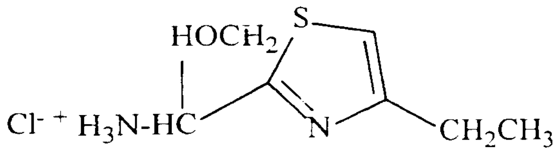
(32)

dehydrogenating **(32)** to produce



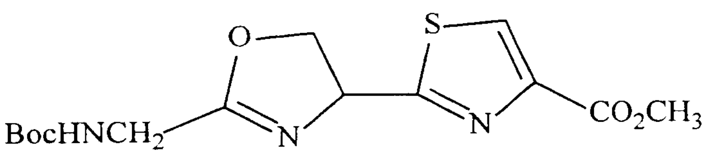
(33)

removing the Boc and Trt protecting groups to produce



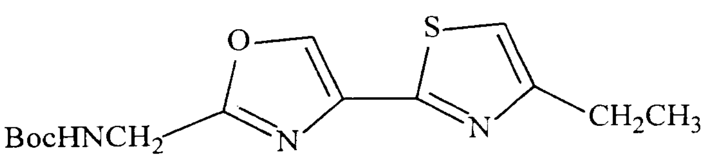
(34a)

effecting a reaction with **(34a)** to produce



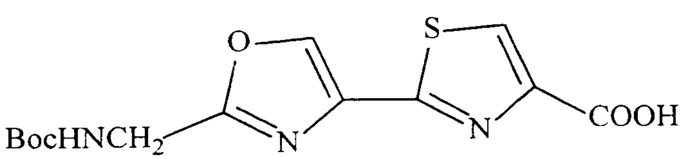
(35)

dehydrogenating **(35)** to produce



(36)

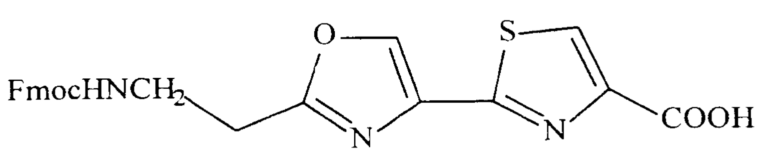
hydrolyzing **(36)** to produce



(37)

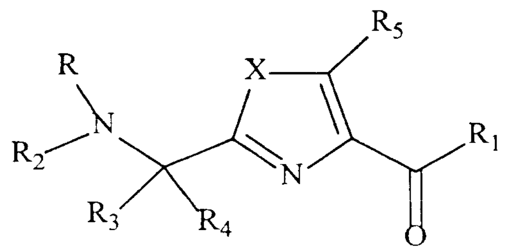
removing the Boc protective group to produce **(3)**.

4. A *N*-protected oxazole and thiazole amino acid comprising the structure of:

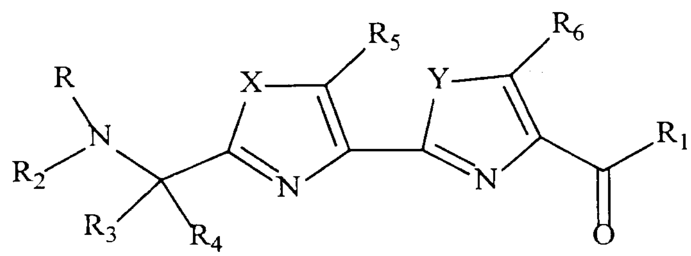


(3)

5. A combinatorial library of at least two compounds, each compound within the library being derived from the solid phase peptide combinatorial synthesis of at least one compound selected from the group consisting of:



(11)



(12)

where R= H, a naturally occurring or synthetic **L** or **D** amino acid, *Tert*-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), carbobenzoxy (Z), Benzozyl (Bz), and other like amino protecting groups;

where R_1 = OH, alkyl esters, aromatic esters such as methyl, ethyl, *t*-butyl and benzyl, activated esters such as pentafluorophenyl, nitrophenyl, N-hydroxysuccinimide, acid chlorides, fluorides, organic salts, such as cyclohexyamines (CHA), amides, an amide bonded to a linker such as a diamine, or an insoluble support for use in solid phase synthesis;

where R_2 = H, a C_1-C_{10} alkyl or an aromatic ring;

where $R_{3\text{-}4}$ = H, or a C_1-C_{10} alkyl;

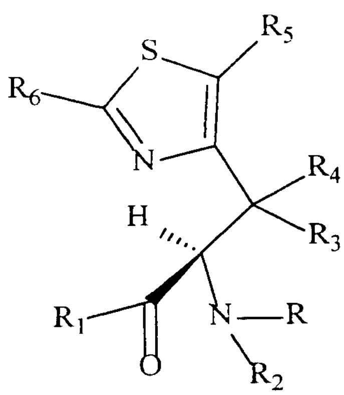
where $R_{5\text{-}6}$ = H, C_1-C_{10} alkyl, a heterocylic ring, an aliphatic or aromatic ring, a functional group such as an amine, an alchohol, a halide or an organometallic complex;

where X = oxygen (O) or sulfur (S);

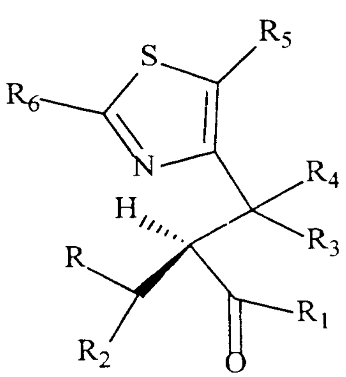
where Y = oxygen (O) or sulfur (S);

wherein at least one of the compounds selected from the group consisting of **11** and **12** forms an amide bond with at least one of the compounds selected from the group consisting of **11** and **12** or a naturally occurring or synthetic amino acid.

6. A combinatorial library of at least two compounds, each compound within the library being derived from the solid phase peptide combinatorial synthesis of a synthetic combinatorial library of at least two compounds, each compound within the library being derived from the solid phase combinatorial synthesis of at least one compound selected from the group consisting of:



(13)



(14)

where R= H, a naturally occurring or synthetic **L** or **D** amino acid, *Tert*-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), carbobenzoxy (Z), Benzozyl (Bz), and other like amino protecting groups;

where R_1 = OH, alkyl esters, aromatic esters such as methyl, ethyl, *t*-butyl and benzyl, activated esters such as pentafluorophenyl, nitrophenyl, N-hydroxysuccinimide, acid chlorides, fluorides, organic salts, such as cyclohexyamines (CHA), amides, an amide bonded to a linker such as a diamine, or an insoluble support for use in solid phase synthesis;

where R_2 = H, a C_1-C_{10} alkyl or an aromatic ring;

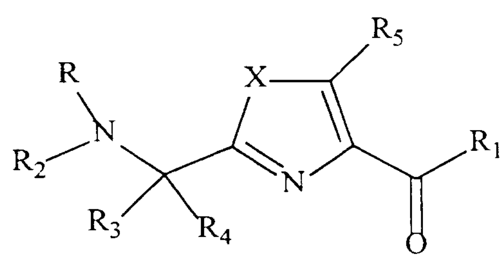
where $R_{3\text{-}4}$ = H, or a C_1-C_{10} alkyl;

where $R_{5\text{-}6}$ = H, C_1-C_{10} alkyl, a heterocylic ring, an aliphatic or aromatic ring, a functional group such as an amine, an alchohol, a halide or an organometallic complex;

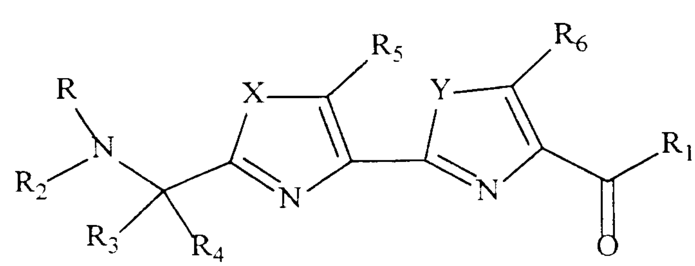
wherein at least one of the compounds selected from the group consisting of **13** and **14** forms an amide bond with at least one of the compounds selected from the group consisting of **13** and **14** or a naturally occurring or synthetic amino acid.

7. A method for the preparation of a library of claim 5 comprising the following steps:

coupling an amino protected first amino acid to a resin, the first amino acid selected from the group consisting of:



(11)



(12)

where R= H, a naturally occurring or synthetic **L** or **D** amino acid, *Tert*-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), carbobenzoxy (Z), Benzozyl (Bz), and other like amino protecting groups;

where R_1 = OH, alkyl esters, aromatic esters such as methyl, ethyl, *t*-butyl and benzyl, activated esters such as pentafluorophenyl, nitrophenyl, N-hydroxysuccinimide, acid chlorides, fluorides, organic salts, such as cyclohexyamines (CHA), amides, an amide bonded to a linker such as a diamine, or an insoluble support for use in solid phase synthesis;

where R_2 = H, a C_1-C_{10} alkyl or an aromatic ring;

where R_{3-4} = H, or a C_1-C_{10} alkyl;

where R_{5-6} = H, C_1-C_{10} alkyl, a heterocylic ring, an aliphatic or aromatic ring, a functional group such as an amine, an alchohol, a halide or an organometallic complex;

where X = oxygen (O) or sulfur (S);

where Y = oxygen (O) or sulfur (S);

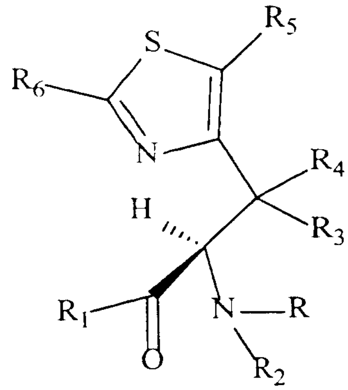
removing the protecting group of the first amino acid;

coupling an amino protected second amino acid selected from the group consisting of **11** and **12** or a naturally occurring or synthetic amino acid; and

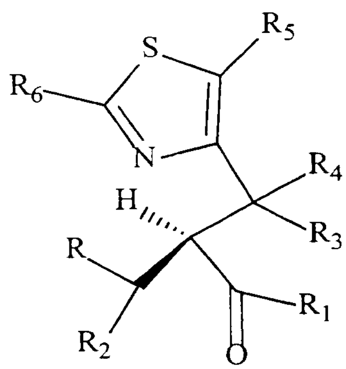
cyclizing the compounds selected from the group consisting of **11** and **12** or a naturally occurring or synthetic amino acid from the step of coupling.

8. A method for the preparation of a library of claim 6 comprising the following steps:

coupling an amino protected first amino acid to a resin, the first amino acid selected from the group consisting of:



(13)



(14)

where R= H, a naturally occurring or synthetic **L** or **D** amino acid, *Tert*-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), carbobenzoxy (Z), Benzozyl (Bz), and other like amino protecting groups;

where R_1 = OH, alkyl esters, aromatic esters such as methyl, ethyl, *t*-butyl and benzyl, activated esters such as pentafluorophenyl, nitrophenyl, N-hydroxysuccinimide, acid chlorides, fluorides, organic salts, such as cyclohexyamines (CHA), amides, an amide bonded to a linker such as a diamine, or an insoluble support for use in solid phase synthesis;

where R_2 = H, a C_1-C_{10} alkyl or an aromatic ring;

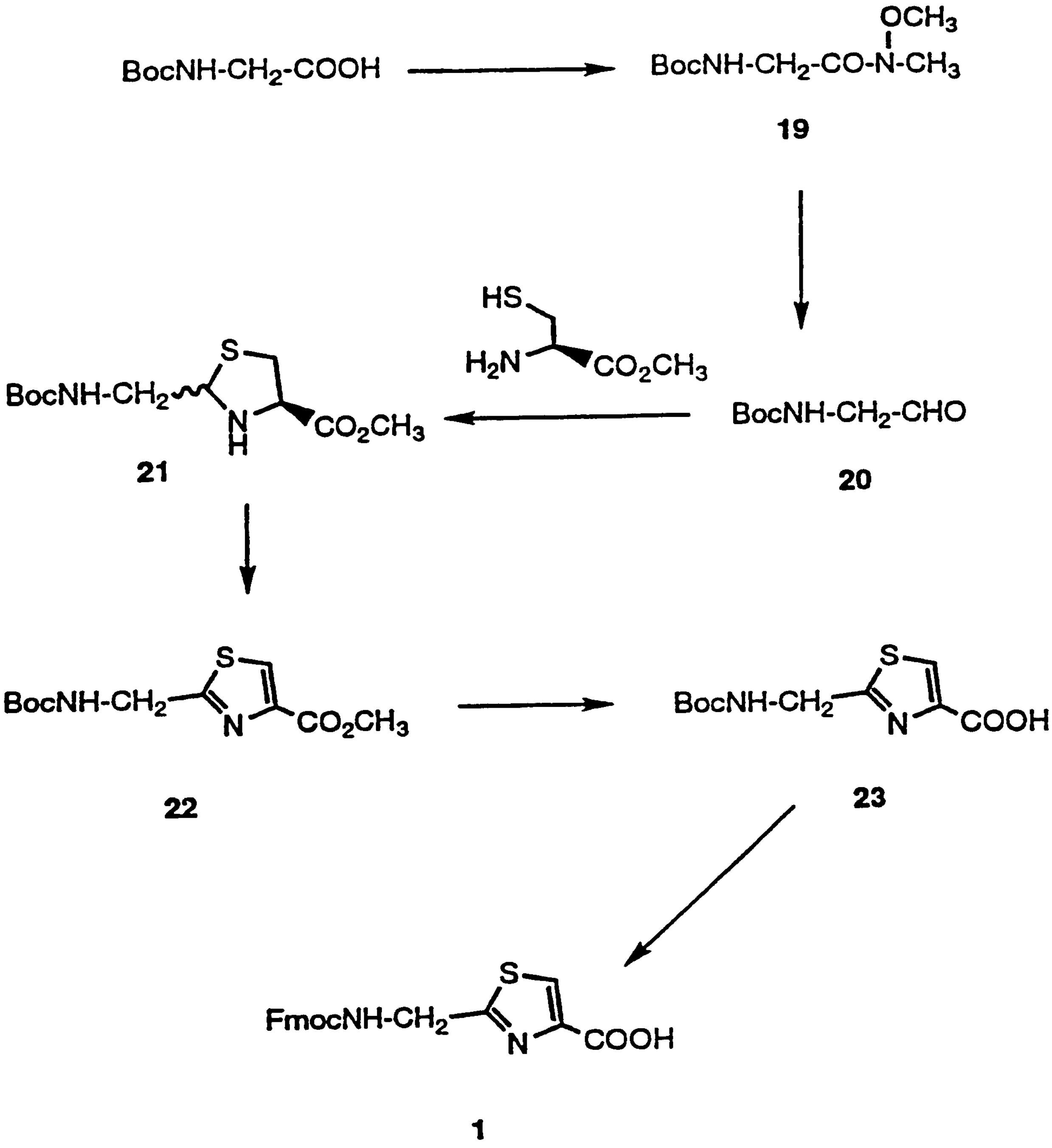
where R_{3-4} = H, or a C_1-C_{10} alkyl;

where R_{5-6} = H, C_1-C_{10} alkyl, a heterocylic ring, an aliphatic or aromatic ring, a functional group such as an amine, an alchohol, a halide or an organometallic complex;

removing the protecting group of the first amino acid.

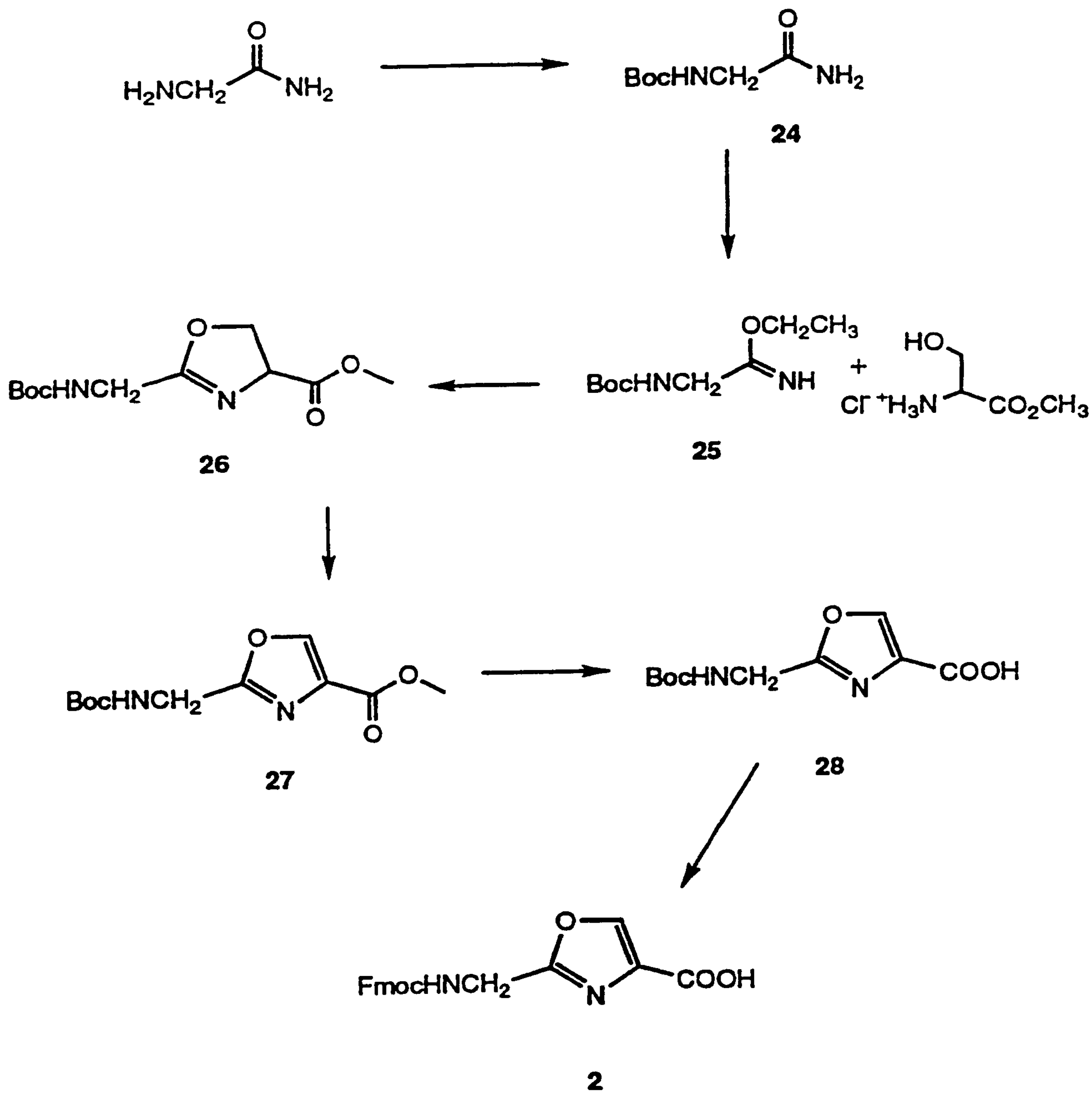
coupling an amino protected second amino acid selected from the group consisting of **13** and **14** or a naturally occurring or synthetic amino acid; and

cyclizing the compounds selected from the group consisting of **13** and **14** or a naturally occurring or synthetic amino acid from the step of coupling.



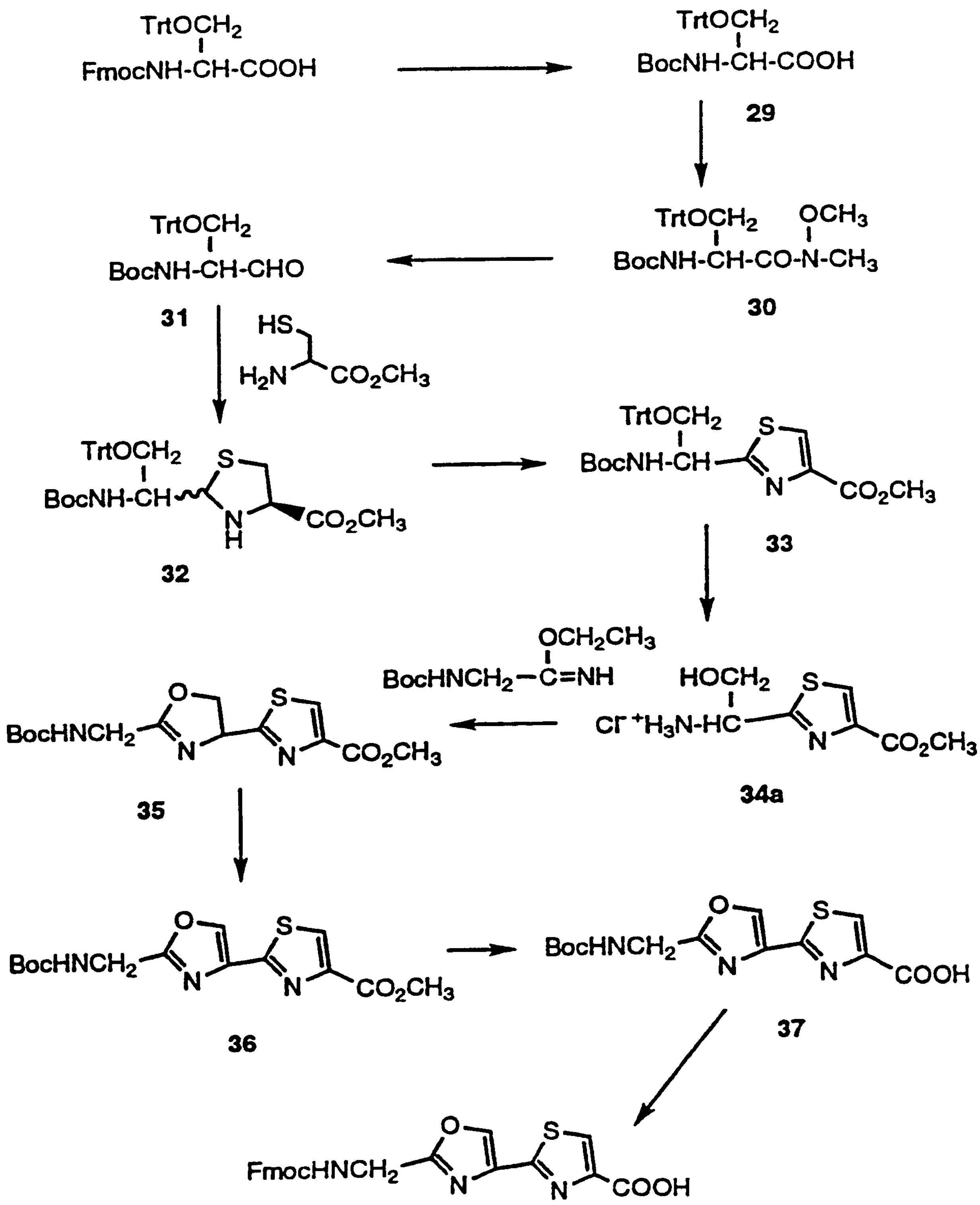
BocNH-CH2-COOH
OCH3
BocNH-CH2-CO-N-CH3
19
HS
H2N
CO2CH3
BocNH-CH2
S
N
H
CO2CH3
21
BocNH-CH2-CHO
20
BocNH-CH2
S
N
CO2CH3
22
BocNH-CH2
S
N
COOH
23
FmocNH-CH2
S
N
COOH
1

FIG. 1



H_2NCH_2
O
NH_2
$BocHNCH_2$
24
OCH$_2$CH$_3$
NH
25
+
HO
$Cl^- {}^+H_3N$
CO_2CH_3
26
27
28
COOH
$FmocHNCH_2$
2

FIG. 2


TrtOCH2
FmocNH-CH-COOH
TrtOCH2
BocNH-CH-COOH
29
TrtOCH2 OCH3
BocNH-CH-CO-N-CH3
30
TrtOCH2
BocNH-CH-CHO
31
HS
H2N CO2CH3
TrtOCH2
BocNH-CH
S
N
H
CO2CH3
32
TrtOCH2
BocNH-CH
S
N
CO2CH3
33
OCH2CH3
BocHNCH2—C=NH
HOCH2
Cl⁻ ⁺H3N-HC
S
N
CO2CH3
34a
BocHNCH2
O
N
S
N
CO2CH3
35
BocHNCH2
O
N
S
N
CO2CH3
36
BocHNCH2
O
N
S
N
COOH
37
FmocHNCH2
O
N
S
N
COOH

3

FIG. 3

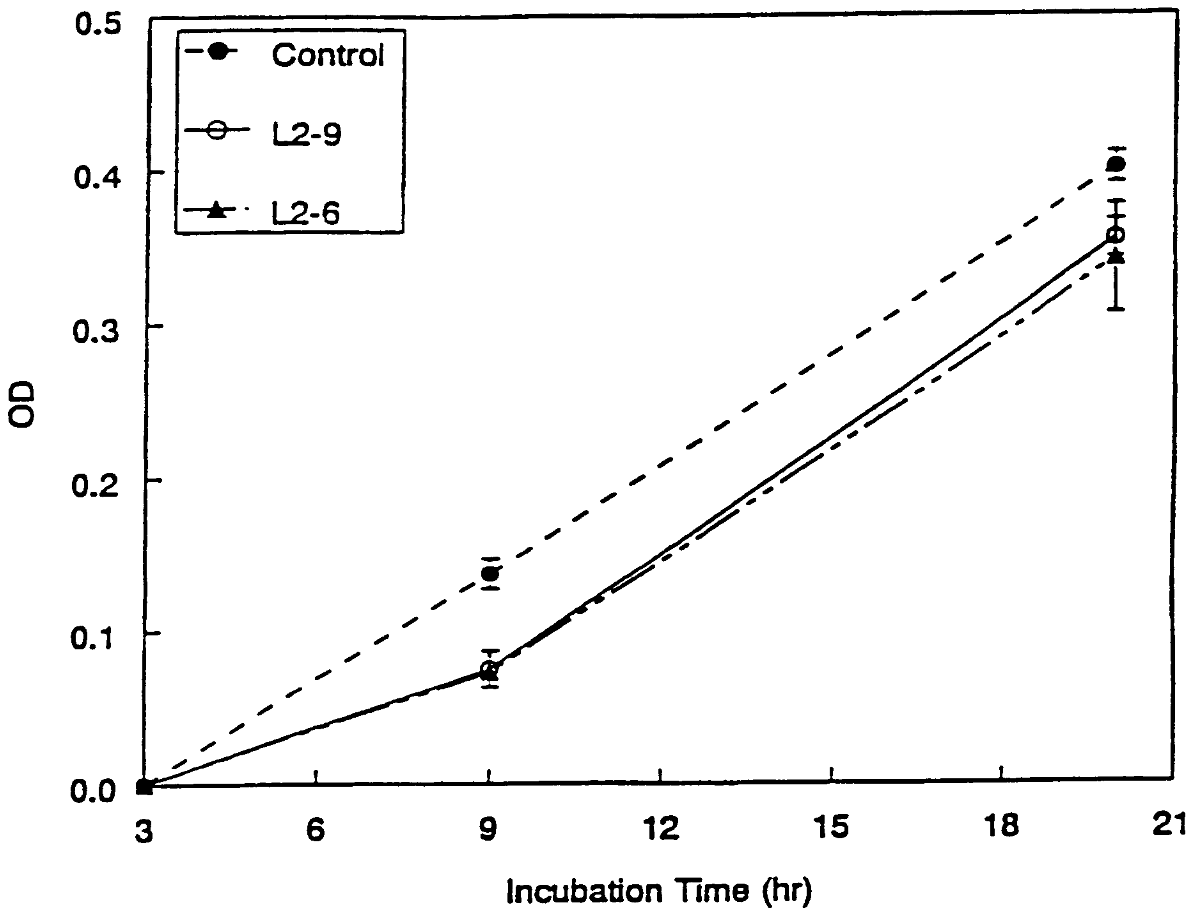

Control
L2-9
L2-6
0.5
0.4
0.3
0.2
0.1
0.0
OD
3
6
9
12
15
18
21
Incubation Time (hr)

FIG. 4

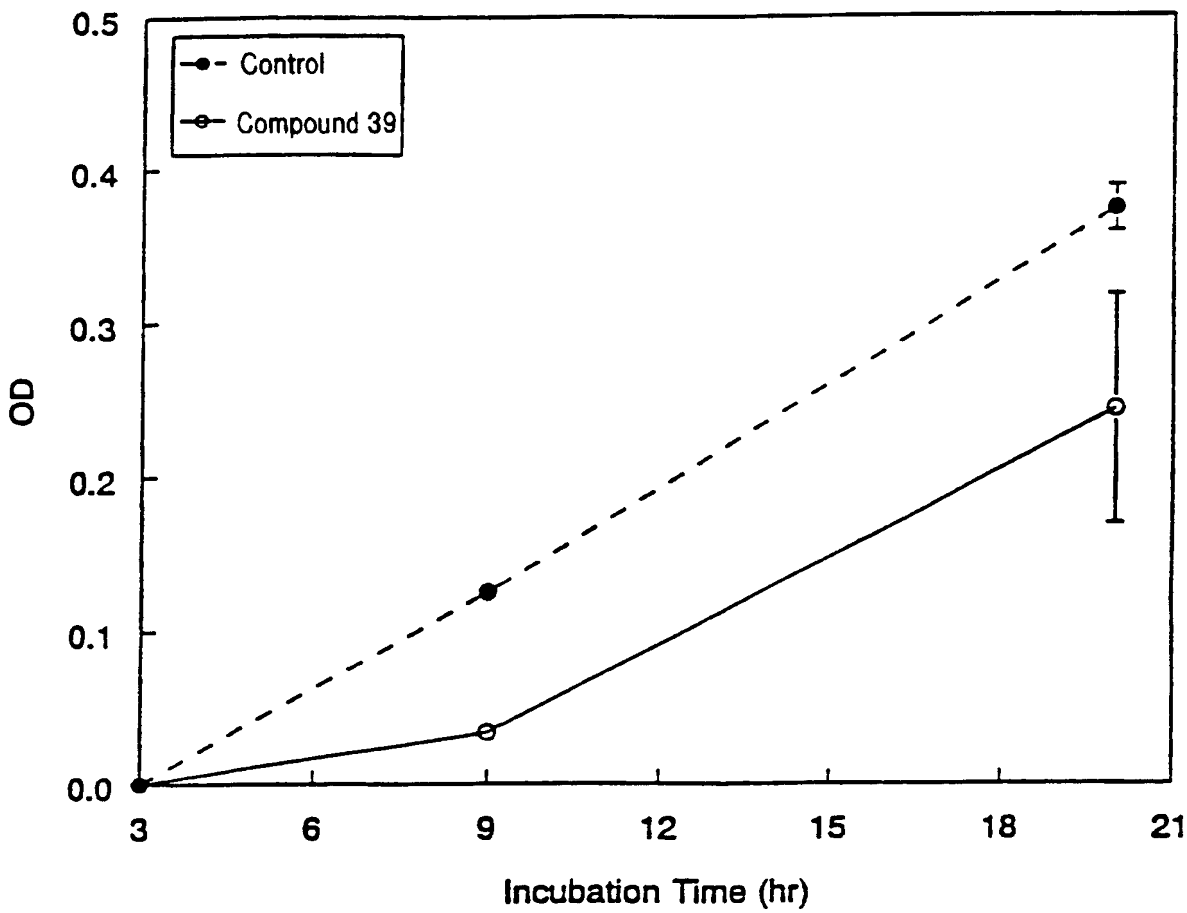

0.5
0.4
0.3
0.2
0.1
0.0
OD
Control
Compound 39
3
6
9
12
15
18
21
Incubation Time (hr)

FIG. 5

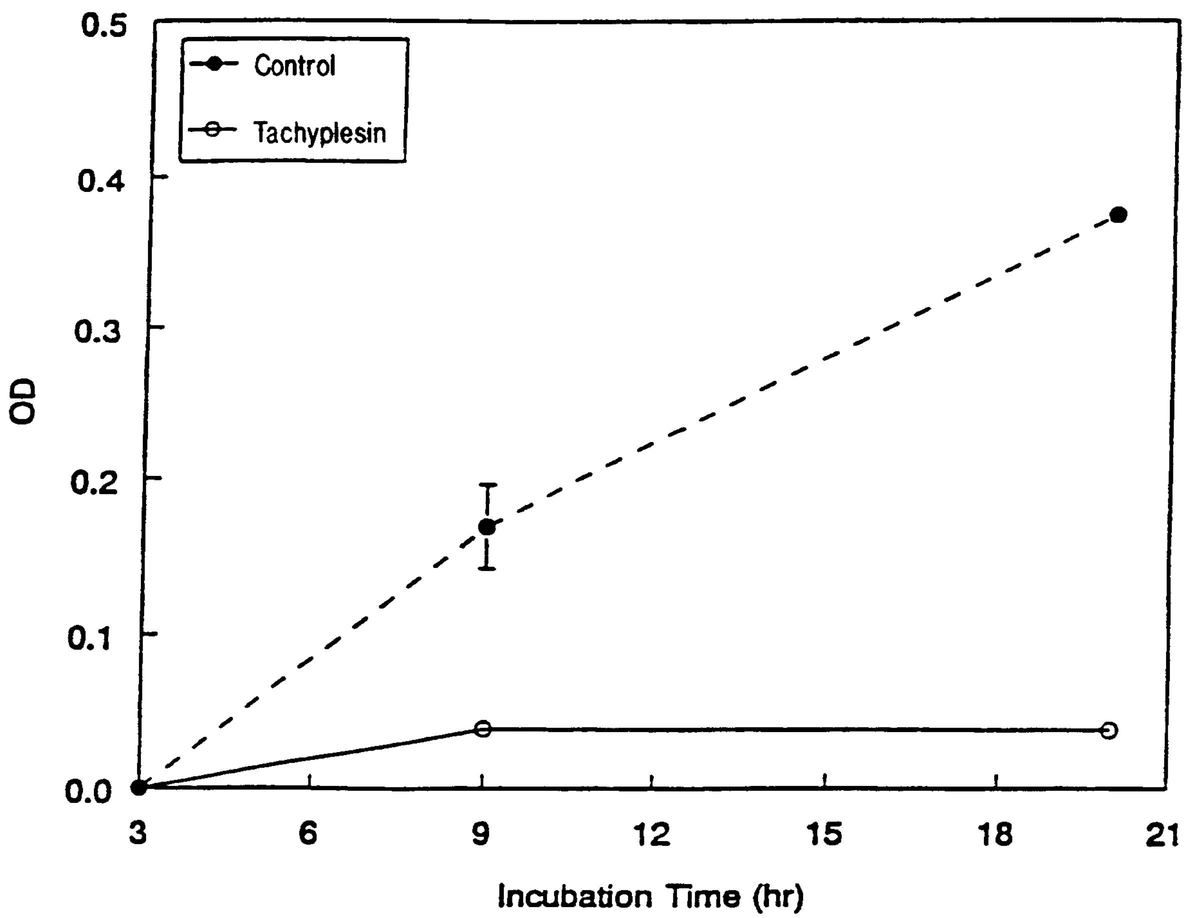

Control
Tachyplesin
0.5
0.4
0.3
0.2
0.1
0.0
OD
3
6
9
12
15
18
21
Incubation Time (hr)

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/07564

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 548/146, 200, 201,202, 203, 204, 205, 214, 235, 236; 435/4, digest 34,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
The Heterocycles (John Weiley and Sons) Volume 34 parts 1-3 and Volume 45.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,164,371 A (EDWARDS et al), 17 Novenmber 1992, see entire document, note that the reference teaches the oxidation of thiazolines to thiasole is known in the art.	1, 7
Y	US 5,589,356 A (TAM) 31 December 1996, see the entire document, note that the reference teaches that aldehydes can be condensed with alpha amino thiols or alcohols to form thiazolidines (Figure 1).	1, 2, 7
Y	US 5,866,387 A (OGINO et al) 02 February 1999, see the entire document, note that the reference teaches the formation of thiazolidines from aldenhydes and alpha amino thiols, these comounds can be oxidized to thiazloes. as per US 5,164,371.	1, 7

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
	27 JUN 2000

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Deila Collensch
Facsimile No.	Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/07564

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,635,502 A (FLYNN) 03 June 1997, see entire document. note that the reference teaches the formation of thiazolidines from an aldehyde and an alpha amino alcohol; see column 7, Scheme B.	1, 7
A	US 5, 847,150 A (DORWALD) 08 December 1998, see entire document, note the libraries of substituted thiazoles.	1, 4, 5, 7
Y	TURCHI, I.J., Synthesis reactions of alkyl-, aryl- and aralkyloxazoles. Heterocyclic Compounds. Oxazoles. New York: John Wiley & Sons. 1986, Vol. 45, pages 15-22, note the syntheis of oxazolidines via an iminoehter and the subsequent oxidation of oxazolidines to oxazoles in sections 1.2.10 and 1.2.15 respectively.	2
X --- Y	WO 95/04277 A1 (SPHINX PHARMACEUTICALS INC.) 09 February 1995, see the entire document, especially the universal library strucure of formula I starting on page 6.	5 --- 7

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-5 and 7

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/07564

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07D 277/04, 277/08, 277/20, 277/22, 277/28, 277/30, 275/02, 263/30, 263/34; C12Q 1/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

548/146, 200, 201,202, 203, 204, 205, 214, 235, 236; 435/4, digest 34,

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

CAS ONLINE (structure search), WEST (US, JPO, EP and Derwent) Oxazole, oxazoline, thiazole, thiazolidine, amino alcohols, iminoether, combinatorial, libraries, synthesis , dehydrogenation and oxidation.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim 1, drawn to a method of producing thiazole compounds.
Group II, claim 2, drawn to a method for producing oxazole compounds.
Group III, claims 3,4, drawn to a method of producing bridged oxazole-thiazole compounds and bridged oxazole thiazole compounds.
Group IV, claim 5, drawn to a combinatorial library of oxazole-thiazole compounds.
Group V, claim 6, drawn to a combinatorial library of stereochemically pure thiazole compounds.
Group VI, claim 7, drawn to a method of preparing a library of group IV.
Group VII, claim 8, drawn to a method of preparing a library of Group V.
The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The compounds and libraries are known in the art hence the structural features which would unite the inventions do not constitute special technical features.